Inflammation and Fibrosis – Interactions and Impact on the Kidney

Agnes B. Fogo, M.D.
Department of Pathology, Microbiology and Immunology
Vanderbilt University Medical Center, Nashville, TN

Introduction
Chronic kidney disease (CKD) is characterized by progressive interstitial fibrosis, tubular atrophy and glomerulosclerosis. The mechanisms of fibrosis are complex, and may involve excess synthesis of collagen with decreased degradation, in association with parenchymal injury and loss of functional tubules and glomeruli. The extracellular matrix (ECM) that makes up the fibrotic kidney has numerous sources, but in particular is contributed to by activated myofibroblasts. Regardless of the type of CKD, inflammatory cells are associated with the interstitial fibrosis. These inflammatory cells are diverse, including macrophages, various T-cells, dendritic cells, plasma cells and even granulocytes in more acute phases of injury. The impact and interaction of these cells with the parenchyma and effect on fibrosis are complex and stage specific.

Monocyte/macrophage subsets
Similar to evolution of our understanding of the complex various subsets of lymphocytes over the last many decades, monocyte/macrophages are now recognized to consist of unique subsets with varying cell surface markers, chemokine receptors and roles in injury and repair vs. fibrosis. Cells with varying expression of the cell surface marker Ly6C show functionally important differences in receptor expression of key chemokines. Monocytes that express Ly6C show high levels of the chemokine receptor CCR2 but low levels of CXCR1 (CCR2<sup>high</sup> CXCR1<sup>low</sup>). In contrast, monocytes without Ly6C on their surface have low levels of CCR2 and high levels of CXCR1. These CCR2<sup>high</sup> CXCR1<sup>low</sup> monocytes are the typical cells that infiltrate tissue and are implicated in disease processes and have been termed inflammatory or M1 macrophages. The Ly6C negative cells (Ly6C-CCR2<sup>low</sup>CXCR1<sup>high</sup> cells) are also termed resident monocytes and may replenish tissue macrophages and dendritic cells. Tissue macrophages also have discrete phenotypes. Classically activated macrophages, M1, and alternatively activated macrophages, M2, were initially distinguished by their responses in vitro to interferon γ (IFN-γ) and lipopolysaccharide for an M1 phenotype, with an M2 phenotype observed after treatment with interleukin (IL)-4 or IL-13. M1 macrophages are proinflammatory and secrete TNF-α, IL-1β, reactive oxygen species and nitric oxide. In contrast, the M2 macrophages are anti-inflammatory and secrete IL-6 and insulin-like growth factor 1. M2 macrophages may promote tissue healing and angiogenesis. Further subcategories of these inflammatory cells are evolving based on in vivo responses.

Various theories for the origin(s) of these subtypes have been put forth, including the Hume hypothesis of one macrophage population that may assume infinite phenotypes, vs. the Geissman hypothesis, postulating that each subpopulation has its own unique precursor, and a third model where sequential subpopulations evolve based on tissue environment and activating stimuli. More recent evidence suggests that in various settings, there is evidence to support each of these possibilities. Examination of
the impact of macrophages on parenchymal cells and ultimate response to injury are
beginning to emerge from experimental models. Examination of specific activation state
and subset of macrophages in human tissue in vivo is still limited. However, overall
glomerular or interstitial macrophage number correlates with worse outcome in various
diseases, including fibrosis and tubular atrophy. More recent studies suggest that this
link may be modulated and macrophage phenotype altered with a beneficial impact on
outcome.

**Activation of macrophages**

When activated by various stimuli, including pathogen associated molecular
patterns (PAMPs), which activate macrophages through Toll-like receptors (TLRs) and
other receptors. Signaling is activated through NF kappa-B and MAP kinase, and
proinflammatory cytokines are produced by macrophages include TNFα, interleukin IL-1
β, IL-12, IL-12, IL-13, and IL-16. Further inflammation and influx of other inflammatory
cells is enhanced by macrophage production of chemokines including MIP-1, MIP-2,
and monocyte chemotactic protein. Increased reactive oxygen species generated from
activated macrophages also may contribute to injury. These activated macrophages
expressed Mac2 (galactin-3) in tissue, along with NOS2 or IL-1 β. In contrast to these
injurious activation patterns, macrophages also can elaborate vascular endothelial
growth factor (VEGEF), transforming growth factor-β (TGF-β), IL-10, angiopoietin 1,
hepatocyte growth factor, fibroblast growth factor 2, which together could promote
angiogenesis, wound healing and anti-inflammatory responses. Macrophages may also
scavenge fragments of injured cells and abnormal matrix and thus promote tissue
remodeling and regeneration following injury. In injury settings where there are no
immune complexes or external pathogens, macrophages may be activated by release
of factors from injured cells, which could serve as ligands for activating receptors on the
monocytes. These danger-associated molecular patterns (DAMPs) are factors that may
bind to Toll-like receptors and also promote macrophage activation. DAMPs include
advanced glycation end products, HMGB1, adenosine and others.

Studies now indicate that macrophage polarization also exists in vivo, and not
just in the artifactual in vitro situation. In addition to M1 and M2 macrophages, a
regulatory macrophage appears in response to the anti-inflammatory cytokine IL-10.
Serum amyloid P, also known as pentraxin-2, or apoptotic cells and immune complexes
may in specific contexts result in a macrophage subpopulation that generates
increased IL10 and thus suppresses further immune responses. These regulatory macrophages
therefore may dampen ongoing response of other infiltrating cells.

**Macrophages, angiotensin and effects on tissue injury**

The consequence of macrophages on tissue injury has been studied in various
ways, with depletion of macrophages by transgenic models where these cells
expressed diphtheria toxin on their surface, or with bone marrow transplant experiments,
or with chemical ablation. The renin angiotensin system is a major activator of
progressive kidney disease, and antagonism and/or inhibition of its actions are the
mainstay of treatment of CKD. We therefore investigated the potential role of the
angiotensin type-1 receptor (AT1R) on macrophages in promoting fibrosis. To this end,
we generated chimeric mice that lacked the AT1a subtype receptor only on
macrophages, by performing bone marrow transplant with AT1a deficient marrow after lethal radiation. We then exposed the reconstituted chimeric mice to injury that induced progressive tubulointerstitial fibrosis, namely unilateral ureteral obstruction (UUO). In mice with intact AT1 receptor on parenchymal cells, but with bone marrow-derived cells with absence of AT1, there was surprisingly increased interstitial fibrosis, although macrophage infiltration was decreased. These AT1 deficient macrophages showed defective phagocytosis, and impaired migratory capacity ex vivo. We postulated that a defective phagocytic scavenging by the AT1 receptor deficient macrophages could contribute to a profibrotic phenotype.

**Macrophages, TGF-β and fibrosis**

When macrophages infiltrate tissue, tissue signaling interaction pathways must be intact for fibrosis to be effected. In addition, numerous therapies that decrease fibrosis, including ARBs, PPAR-γ agonists, decrease fibrosis and macrophage infiltration in parallel. Of note, inhibition of TGF-β may have complex actions. In addition to promoting macrophage infiltration and matrix synthesis, TGF-β has important immune modulatory effects. We found that inhibition of TGF-β with a pan-antibody decreased fibrosis at low doses, but higher doses were ineffective, linked to higher levels of infiltrating macrophages.

TGF-β circulates in latent form. Activation of TGF-β by cleaving it from latency-associated peptide can be mediated by e.g. thrombospondin-1 and the integrin αV β6. αV β6 integrin is expressed in epithelium in the lung, the kidney and the skin. β6 deficient mice were protected from lung fibrosis and interstitial fibrosis induced by UUO. Of interest, infiltrating macrophages were even more abundant in β6 knockout mice, but no increase in collagen content was observed after UUO, and TGF-β was not activated by UUO in these mice. However, activation of other pathways by adding exogenous angiotensin II, could restore fibrosis but through a non-TGF-β-dependent pathway, which we linked to increased plasminogen activator inhibitor-1 (PAI-1) and thymosin β4. Thymosin β4 is a G-actin sequestering protein with numerous effects on cell migration, angiogenesis, and activates PAI-1 and TGF-β. We showed that thymosin β4 is necessary for angiotensin to induce PAI-1 in endothelial cells. PAI-1 could influence fibrosis in part by effects on cell migration through vitronectin, and in part by inhibiting plasmin-dependent proteolysis of accumulated ECM proteins. Thymosin β4 promotes wound healing in skin and corneal wounds, by promoting cell migration and ECM and angiogenesis. In early stages after UUO, thymosin β4 increased injury, but at later stages thymosin β4 was protective. Our most recent data with conditional knockout of thymosin β4 in macrophages supports that expression of thymosin β4 may influence polarization of macrophages.

**Modulating macrophages to decrease fibrosis**

Additional recent exciting studies from the laboratory of Duffield shows that treatment with exogenous pentraxin-2 (serum amyloid P) also is anti-fibrotic in the UUO model. The postulated mechanism may be opsonization of cell debris with serum amyloid P, and interaction with activated Fcy receptors on macrophages, resulting in an anti-inflammatory IL10 expression. These and other studies have led to the theoretical consideration of yet another macrophage population, a potentially fibrolytic macrophage.
In additional studies, mice were genetically manipulated to express the diphtheria toxin only on macrophages, under the CD11b promoter. Injection of the toxin then resulted in specific monocyte/macrophage depletion. Ablation of monocyte/macrophages at day 4 through 6 after UUO resulted in decreased fibrosis. Similar results were seen when macrophage ablation was induced at a later stage after UUO from day 7 to 10. Fibrosis could be restored by adoptive transfer of monocytes from normal mice. Interestingly, when resident renal macrophages were depleted there was no effect on fibrosis- only depletion of circulating monocytes and recruited macrophages blunted fibrosis. In the nephrotoxic serum nephritis model, macrophage ablation also decreased injury, with decreased interstitial myofibroblasts and fibrosis. Of note, depletion of macrophages from mice with skin wounds resulted in decreased ECM deposition and cell proliferation and delayed healing. These mechanisms were tied to interference with macrophage production of PDGF, which in turn led to decreased activation of fibroblasts and fibroblast osteopontin expression. A balance of macrophage effects therefore appears to be important in early wound healing.

A key step in macrophage modulation of fibrosis is modulation of monocyte recruitment to become tissue macrophages. The monocyte chemoattractant protein (MCP-1, or CCL2) and its receptor CCR2, are key for activation and chemotaxis. In mice genetically deficient in CCL2, macrophage infiltration was decreased and fibrosis was diminished in models of UUO and early diabetic injury. However, the specific phenotypes of resulting macrophages were not explored. Similarly, CCR2 receptor inhibitors ameliorated renal fibrosis. Interestingly, blockade of CCR1, which is present on M2 macrophages, decreased macrophages and fibrosis in the Alport mouse model, but CCR2 blockade was ineffective, while CCR1 blockade was effective in db/db mice, adriamycin nephropathy and UUO.

In addition to experimental maneuvers to deplete macrophages, infusion of modified macrophages has been examined for potential therapeutic impact on fibrosis. The impact of modulating macrophages will likely vary depending upon whether it is initiated in the injury or healing phase. When macrophages were transferred to mice at a late stage after UUO, there was attenuation of fibrosis. Manipulation of specific cell populations will influence the responsiveness. For instance, depletion of dendritic cells selectively did not affect renal fibrosis, while depletion of all monocyte lineage cells could have this beneficial effect in the UUO model. Specific injection of M2 macrophages primed with IL-4 and IL-13 ameliorated adriamycin nephropathy and reduced endogenous macrophage infiltration, whereas injection of M1 macrophages worsened injury and increased inflammatory gene expression, and unmodified macrophages did not alter the disease course.

In addition to promoting or regulating angiogenesis and myofibroblast production of matrix, macrophages have effects on parenchymal cells. Macrophages can promote activation to cell cycle entry and death in injured parenchymal cells. Entry of injured cells into cell cycle may lead to their death by pausing at DNA checkpoints and thus promoting apoptosis whereas cells with intact functionality will be able to continue through cell cycle without cell death.

Galactin-3 macrophage expression was necessary for them to transduce fibrosis. Interestingly, the kidney parenchyma itself may be important in mediating the renal...
accumulation of specific macrophage phenotypes. The transcription factor Krüppel-like factor-5 (KLF-5) is expressed mostly in collecting duct epithelial cells, and haplo-insufficient mice (KLF-5 +/-) had less injury after UUO than wild type, with decreased macrophage infiltration of the M1 phenotype, and increased accumulation of M2. KLF-5, in concert with C/EBPα, mediated expression of chemotactic proteins S100a8 and S100a9, resulting in recruitment of inflammatory monocytes to the kidney and their activation into M1 type macrophages. This collecting duct-derived transcription factor thus played a major role in influencing the phenotype and consequences of inflammatory cell infiltration.

Other parenchymal receptors also contribute to tissue inflammation. Discoidin domain receptor-1 (DDR-1) is a tyrosine kinase transmembrane receptor for collagen constitutively expressed in several organs including the kidney, where it is found predominantly on vascular smooth muscle cells, mesangial cells and epithelial cells. DDR-1 is activated by binding to collagens I to VI and VIII and regulates cell differentiation, adhesion, proliferation and ECM remodeling. It is upregulated after UUO. DDR-1 deficient mice had decreased collagen and TGF-β and remarkable decrease in macrophages, but no shift in M1/2 polarization.

Summary

In summary, macrophages show diverse phenotypes that are context dependent. The ultimate effect on response to injury depends upon balance of macrophage phenotypes and their persistence or resolution, and whether injury and inflammatory stimuli persist or resolve. Inflammatory macrophages may play beneficial roles in response to transient inflammation with induction of macrophages that can suppress further inflammatory response and promote repair. Later in wound healing, profibrotic macrophages promote fibroblast proliferation and fibrogenesis with elaboration of TGF-β. Macrophages may also promote clearance of ECM, through the postulated fibrolytic macrophage phenotype. Anti-inflammatory M2 macrophages may promote tubular repair.
References:


Conway B and Hughes J: Cellular orchestrators of renal fibrosis. QJM 2011 (epub head of print)


