



Macrophage diversity in renal injury and repair

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Monocyte-derived macrophages can determine the outcome of the immune response and whether this response contributes to tissue repair or mediates tissue destruction. In addition to their important role in immune-mediated renal disease and host defense, macrophages play a fundamental role in tissue remodeling during embryonic development, acquired kidney disease, and renal allograft responses. This review summarizes macrophage phenotype and function in the orchestration of kidney repair and replacement of specialized renal cells following injury. Recent advances in our understanding of macrophage heterogeneity in response to their microenvironment raise new and exciting therapeutic possibilities to attenuate or conceivably reverse progressive renal disease in the context of fibrosis. Furthermore, parallels with pathological processes in many other organs also exist.

Macrophages comprise a heterogeneous population of cells that belong to the mononuclear phagocyte system. They play an important role in tissue homeostasis and remodeling and are also potent immune regulators. Although widely recognized as contributing to the pathogenesis of renal fibrosis, glomerular and interstitial macrophages may also play beneficial, reparative, and matrix remodeling roles during tissue repair. There is compelling evidence that macrophages actively participate in the resolution of injury and promote tissue restoration in both immune- and non-immune-mediated renal disease.

The heterogeneity of macrophages, their diverse roles in inflammation and tissue remodeling, and the coordinated activation and programming by other inflammatory cells is not fully understood. Functionally distinct subpopulations of macrophages, together with dendritic cells, may exist in the same tissue and play critical roles in both the initiation and recovery phases of scarring. The origin and activation state of the macrophage and the microenvironment in which they reside are critical determinants of their response to injury. Macrophages that secrete antiinflammatory cytokines, promote angiogenesis, and play a positive role in wound healing and tissue remodeling have been generally referred to as possessing an “alternative” phenotype. They are renowned for their heterogeneity and plasticity, which are reflected by their specialized functions in tissue inflammation and resolving injury. Macrophages have the ability to fuse with themselves and other cell types, particularly in response to inflammatory stimuli. Macrophages may therefore provide an important link between the bone marrow compartment and the regeneration of specialized cells of the kidney and other organs. This review discusses the heterogeneity of macrophages, their activation states, and diverse roles ranging from renal inflammation and replacement of damaged and apoptotic cells, to tissue remodeling. Fundamental insights into the therapeutic application of these antiinflammatory and reparative macrophage functions to renal diseases are discussed.

Macrophage origin and heterogeneity

Macrophages are the oldest cell type in the hematopoietic system. Modern-day mammalian macrophages bear resemblance to the amoebocytes in the circulation of horseshoe crabs (*Limulus* spp.) and have remained largely unchanged for millions of years (1). During early mammalian development, primitive macrophages appear to arise from a different cellular origin distinct from the blood monocyte (2–6). These primitive fetal macrophages have a high proliferative capacity and are derived from PU.1-negative hematopoietic cells (PU.1 is a tissue-specific transcription factor that is expressed in cells of the hematopoietic lineage) that lack monocytic cell surface markers (6–8). Apart from their role in the clearance of dying cells (9), fetal macrophages play a trophic role in promoting organ growth and nephrogenesis in the developing kidney (10). Once permanent or definitive hematopoiesis is established, the proliferative capacity of the macrophage declines and a distinct set of phagocytes, the monocyte-macrophages, are formed (6, 7, 11).

Circulating monocytes derived from common bone marrow myeloid progenitors demonstrate a high cellular plasticity and can form tissue macrophages and dendritic cell subsets through a transdifferentiation process (8, 12, 13). In addition, monocytes can differentiate into osteoclasts, which are fused polykaryons, as a result of an M-CSF- or RANKL-dependent cell-cell fusion process (13, 14). Monocytes themselves demonstrate antigenic and functional heterogeneity dependent on steady state or inflammatory cues. The recruitment of CCR2⁺Ly6⁺ monocytes to sites of inflammation confirms that specific monocyte subsets are involved in an immune response or tissue remodeling (15). Sunderkotter et al. (16) reported that distinct subsets of monocytes distinguished by differential expression of Ly-6C may represent different stages of a continuous maturation pathway. Furthermore, a common monocyte progenitor characterized as CX3CR1⁺CD117⁺Lin⁻ has been described that can selectively differentiate into macrophage subsets and resident spleen dendritic cells (17).

During enhanced recruitment in response to disease states, inflammatory monocytes are recruited in response to cytokine cues and undergo differentiation into two broad but distinct subsets of macrophages that are categorized as either classically activated (M1) or alternatively activated (M2). M2 macrophages represent various phenotypes that are further subdivided into M2a (upon exposure to IL-4 or IL-13), M2b (induced by immune com-

Nonstandard abbreviations used: AT1, angiotensin II receptor type 1; MCP-1, monocyte chemoattractant protein 1; SHIP, Src homology 2-containing inositol-5'-phosphatase; Ym-1, chitinase 3-like 3.

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Table 1
Macrophage activation states and functions

Macrophage phenotype	Activation state	Stimuli	Phenotypic function	Cytokine and inflammatory profile	Unique surface markers
M1	Classical activation	IFN- γ + LPS, TNF, GM-CSF, TLR/IL-1R ligand	Proinflammatory Th1 response	IL-1, IL-12, IL-15, IL-18, IL-23, TNF- α , IL-6, MCP-1, CCL2, CCL3, CCL4, CCL20/MIP-3a, ROS, NO, iNOS, NOS2	CD86, CD80, MHC class II ^{hi} , IL-1R, IL-12 ^{hi} , IL-23 ^{hi} , IL-10 ^{lo}
M2	Alternate activation (M2a polarization)	IL-4 or IL-13	Th2 responses, type II inflammation	Fibronectin, BIG-H3, arginase-1, TNF- α , IL-6, IGF, CCL13/MCP-4, CCL22, CCL18, β 2 integrins	Mannose receptor, scavenger receptor, MHC class II ^{hi} , decoy IL-1R11, FIZZI/Ym-1
	Type II activation (M2b polarization)	Immune complex + TLR/IL-1R ligands	Immunoregulation, Th2 activation	IL-10, TNF- α , IL-1, IL-6, IL-12, SPHK1, CCL1	CD86, MHC class II ^{hi} , IL-10 ^{hi} , IL-12 ^{lo}
	Deactivated (M2c polarization)	IL-10, TGF- β , glucocorticoids	Immunosuppression, matrix remodeling, tissue repair	IL-10, IL-1 β , IL-6, TGF- β , ECM proteins, CCL16, CCL18, arginase-1	SLAM (CD150), mannose receptor, MHC class II ^{lo}

BIG-H3, fasciclin domain 4 protein; MIP, macrophage inflammatory protein; SLAM, signaling lymphocytic activation molecule; SPHK1, sphingosine kinase 1. Note that *FIZZI* and *Ym1* gene expression is characteristic of the alternative pathway of macrophage activation.

plexes in combination with IL-1 β or LPS), and M2c cells (following exposure to IL-10, TGF- β , or glucocorticoids) (18, 19), as detailed in Table 1. The plasticity and differentiation of macrophages into M1 and M2 functional phenotypes therefore represent extremes of a continual spectrum of differential pathways.

Activation of M1 macrophages by classical immune pathways involves an IFN- γ -dependent Th1-type response. Exposure to IFN- γ and LPS or cytokines TNF and GM-CSF induces M1 polarization that is characterized by the production of IL-12 and IL-23, both known to be produced by APCs. The capacity of macrophages and dendritic cells to produce IL-12 and IL-23 strongly influences the outcome of the Th1, Th17, and CD4⁺ T cell response. In addition to Th1 cells, IL-23 and Th17 cells play an important role in the pathogenesis of autoimmune disorders and renal allograft (20). In addition, neutralizing anti-IL-12/23p40 antibodies have been used successfully to treat psoriatic skin inflammation (21, 22). This is a fundamental response paradigm in cellular immunity and in delayed-type hypersensitivity responses that cause tissue damage. For example, in the kidney these processes mediate crescentic glomerulonephritis and acute allograft rejection. In the classical M1 pathway, activation by IFN- γ is crucial; together with a microbial trigger, it induces expression of MHC class II antigens and proinflammatory cytokines.

The alternative M2 macrophage activation pathway typically deactivates macrophages after exposure to Th2-type cytokines. Such responses characterize immunoregulatory, immunosuppressive, and protumoral settings (19). M2 cells induced by exposure to IL-4 and IL-13 (M2a) and deactivating cytokines such as IL-10 and TGF- β (M2c) are thought to suppress immune responses and promote tissue remodeling (19, 23–26). A role for the NF- κ B activator IKK β has also been implicated in the promotion of an alternative immunosuppressive phenotype (27). Macrophage reactivation during antiinflammatory responses may occur as a consequence of innate or acquired immune responses, often characterized by macrophage uptake of apoptotic cells or lysosomal storage of host molecules. Unlike its classically activated counterpart, the M2 macrophage may help to resolve inflammation through high endocytic clearance capacities and production of trophic factors,

together with reduced proinflammatory cytokine secretion (28). For example, M2 macrophages may secrete trophic factors that promote angiogenesis and mediate wound healing by promoting ECM remodeling (29). M2 macrophages also express fibronectin 1 (FN-1), the TGF- β -induced matrix-associated protein BIG-H3, and IGF-1, which provide signals for tissue repair and proliferation. (25). They can generate arginase-1, which suppresses inflammation by inhibiting the production of proinflammatory NO (29). Furthermore, M2 cells express the IL-1 receptor antagonist, which inhibits the effects of the proinflammatory cytokine IL-1, the mannose receptor, and chitinase 3-like 3 (Ym-1) (30). It has recently been reported that a subset of adipose tissue macrophages exhibiting an M2 phenotype produce MMP-9, which in the kidney may contribute to attenuation of fibrotic lesions (31).

As a key component of the inflammatory response that determines tissue destruction or recovery, increasing evidence suggests that macrophages do not remain committed to a single activation state. They may regress to a resting state that can subsequently be reactivated another way. Following phagocytosis of apoptotic cells, classically activated M1 macrophages may revert to an M2 activated state (32). Tumor-associated macrophages (TAMs) also provide evidence of a bidirectional transformation between antiinflammatory and immunosuppressive phenotypes (33, 34). Stemming from a common myeloid precursor, there are distinct subpopulations of monocyte-derived TAMs with different functional phenotypes dependent on the site of tumor origin and progression of disease. TAMs share a phenotype similar to that of M2 macrophages and are likely to represent a unique myeloid cell differentiation program (35). Tumor-conditioned granulocytes may play a role in priming macrophages toward either an M1 or M2 phenotype (36).

Reparative role of macrophages in tissue remodeling

Inflammatory cues within the regional microenvironment can prime macrophage phenotype and determine whether these cells will have a beneficial or deleterious effect during tissue repair and remodeling. Macrophage phenotype and function ultimately determine the outcome of inflammation and the development of irreversible tissue scarring (Figure 1). Unselective macrophage depletion, via the admin-

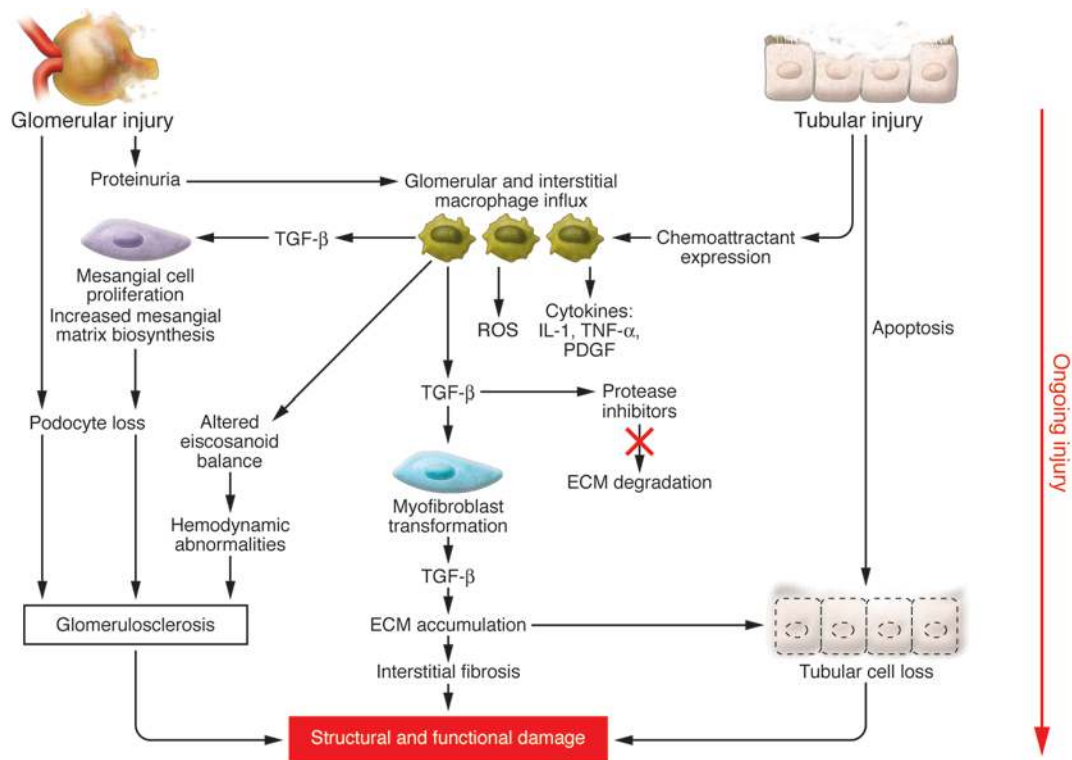


Figure 1

The relationships between infiltrating macrophages and macrophage-derived products in chronic ongoing inflammation lead to structural and functional renal damage. In response to tubular and glomerular injury/dysfunction, macrophage chemoattractants and proteinuria promote the infiltration of renal macrophages, leading to the generation of proinflammatory cytokines, vasoactive eicosanoids, and ROS. The initial injury and proinflammatory state may lead to podocyte and tubular cell apoptosis. Overproduction of TGF- β by macrophages, myofibroblasts, and mesangial cells promotes increased synthesis of glomerular and interstitial ECM proteins and decreased matrix turnover due to the synthesis of matrix-degrading protease inhibitors. The net effect of interstitial fibrosis and/or glomerulosclerosis and podocyte and tubular cell loss is the disruption to tissue architecture and loss of renal function.

istration of anti-macrophage serum or liposomal clodronate, can reduce experimental acute kidney damage by abrogating persistent inflammation and the subsequent development of fibrosis (37–39). Complete macrophage depletion by sublethal irradiation prevents the influx of macrophages to an injured kidney and decreases fibrosis severity (40, 41). However, a selective approach to macrophage depletion will provide greater insight into the role of functionally distinct subpopulations of macrophages that contribute to injury inducing and tissue remodeling phases of inflammatory scarring. Conditional macrophage depletion based on transgenic expression of diphtheria toxin highlights the importance of scar-associated macrophages for recovery responses such as matrix degradation following liver injury (42). Hepatic macrophages were shown to be essential for matrix regression during the recovery phase of experimental hepatic fibrosis (42) and to regulate stellate cell proliferation (43). Within muscle, macrophages enhance myogenic growth by releasing trophic factors that stimulate myogenic precursor cells (44). Using in vivo tracing methods, Arnold et al. (45) showed that CX3CR1^{hi}Ly-6C⁺ inflammatory macrophages initially recruited into skeletal muscle are able to rapidly switch to an antiinflammatory M2 phenotype in response to their changing microenvironment.

Macrophages and their trophic factors are implicated in injury resolution and cellular restoration in a number of organs (46–49). During *Schistosoma* spp. infection, M2 macrophages attenuate

organ injury by downregulating inflammation, predominantly the Th1 response (50). The effect of M2 skewing has been investigated using Src homology 2–containing inositol-5′-phosphatase–null (SHIP-null) mice (51). SHIP is essential for endotoxin tolerance; it dampens LPS-induced M1 activation of bone marrow–derived macrophages. Macrophages from SHIP-null mice manifest an M2 phenotype with constitutively high arginase 1 and Ym-1 levels and require a TGF- β –rich environment during differentiation.

Opposing roles of macrophages in kidney disease and repair

Macrophages promote renal fibrosis, and most interstitial and glomerular renal diseases are characterized by macrophage accumulation. Traditionally, these macrophages are considered transients that enter glomerular or interstitial areas to modulate immune responses and/or process debris and apoptotic cells generated as a result of the primary kidney insult. However, in the face of ongoing injury, sustained macrophage infiltration may result in the continuous production of various wound-healing growth factors. Ultimately this initial process of wound healing becomes pathological, resulting in irreversible fibrosis, tissue destruction, and progressive chronic kidney disease (Figure 1). What begins as an initially essential and beneficial influx of macrophages transforms into their extended presence with damaging consequences.



The progression of immune-mediated renal disease involves an interplay between infiltrating T cells, dendritic cells, and macrophages, which contributes to the immunopathogenesis of glomerulonephritis (52–54). T cell activation is dependent on dendritic cells in secondary lymphoid organs and resident dendritic cells in the kidney (8, 52, 55). Renal allograft rejection is a T cell-dependent process resulting in graft injury through cytotoxic mechanisms and by T cell activation and macrophage effector function (56, 57). Tipping and Holdsworth (58) identified a crucial role for CD4⁺ Th1 cells and macrophages in a delayed-type hypersensitivity mechanism of crescent formation in experimental glomerulonephritis. T cell responses can initiate hypersensitivity immune reactions and stimulate macrophages to generate proinflammatory mediators of injury. During the ensuing damage, macrophages are also the predominant cell type responsible for the development of fibrosis and progressive fibrotic scarring.

Members of the TGF- β superfamily are the most extensively studied macrophage-derived growth factors that have been linked to renal fibrosis (59). Macrophages, tubular epithelial cells, and myofibroblasts are all capable of synthesizing TGF- β at different stages during the development of renal fibrotic lesions (60). However, the observation that macrophage ablation markedly attenuates fibrosis in various conditions suggests that these cells are among the main producers of this growth factor (40, 41). Once activated, TGF- β signals through transmembrane receptors that activate the Smad proteins that regulate the transcription of important target genes including those that encode collagens. In the kidney, macrophage-derived TGF- β may promote fibrosis by paracrine activation of matrix-producing myofibroblasts and promotion of tubular epithelial cell transdifferentiation into myofibroblasts (61, 62). The downstream accumulation of TGF- β -induced ECM is generally considered destructive in nature (63). However, macrophages may synthesize and secrete collagens themselves (64). TGF- β can also deactivate macrophages (65) and induce a tissue-stabilizing, antiinflammatory macrophage phenotype characterized by production of collagen type VI (66).

Modulating macrophage phenotype and function has been reported to reduce renal injury in models of renal disease, including glomerulonephritis (67, 68), allograft injury (69), and interstitial fibrosis (70). In a model of obstructive nephropathy, Nishida et al. (71) showed that angiotensin II receptor type 1-expressing (AT1-expressing) macrophages had a protective effect in later stages of fibrotic injury. Transplantation of bone marrow from AT1-null mice into wild-type mice led to more severe interstitial fibrosis despite reduced numbers of monocytes and macrophage progenitors, compared with mice reconstituted with AT1-positive wild-type bone marrow (71). Similar detrimental effects have been reported when obstructive nephropathy was induced in mice lacking the classical urokinase receptor (uPAR) compared with wild-type mice (72).

Wang et al. (73) provided direct evidence that *ex vivo* manipulation of macrophages can reduce renal injury and facilitate repair by using adoptive transfer studies of M2-polarized macrophages injected into mice with chronic inflammatory renal disease. Splenic macrophages stimulated with IL-4/IL-13 were injected systemically after the onset of disease, where they were found to downregulate inflammatory cytokine and chemokine expression of the host infiltrating macrophages (73). Therefore, the protective effect of the transplanted macrophages was associated with M2-skewing of host macrophages, supporting findings in models of glomerular

disease (74, 75). In a rat model of nephrotoxic nephritis, the transfer of macrophages transduced with an NF- κ B inhibitor resulted in a reduction in iNOS and MHC class II expression in glomeruli (74). Although the injected antiinflammatory macrophages comprised only 15% of the glomerular macrophages, they significantly reduced glomerular infiltration and activation of host macrophages, resulting in the attenuation of renal injury (74).

Bone marrow-derived cells in kidney repair

The kidney has a remarkable ability to regenerate following acute injury. Most notably, the renal epithelia have the intrinsic capacity to rapidly self-duplicate (76). Renal injury and repair comprise a delicate balance between cell loss and proliferation and macrophage-dependent interstitial matrix accumulation and remodeling. Whether bone marrow-derived cells and/or infiltrating macrophages can contribute directly to the replacement of injured and dying tubular epithelia and glomerular cells by transdifferentiation and/or cell-cell fusion is an active area of investigation (Figure 2).

Bone marrow-derived cells are thought to cross lineage boundaries and transdifferentiate, resulting in a phenotype switch in response to inflammatory cues to repair injured organs, including the kidney (77–80). Cell-tracing studies using either Y chromosome tracking in sex-mismatched human kidney transplants (81–83) or GFP⁺ reporter mice (72, 84, 85) provide evidence that bone marrow-derived cells can replace the renal vasculature and interstitial cells (81, 84, 85), renal tubular epithelial cells (77, 82, 83), and cells of the glomerulus (77). Masuya et al. (86) showed that a single hematopoietic cell was capable of differentiating into mesangial cells in lethally irradiated recipient mice. In all studies, hematopoietic engraftment of the host is needed before bone marrow cell engraftment into the kidneys occurs, suggesting that it is the hematopoietic progeny of bone marrow-derived cells and not the stem cells themselves that engraft into host tissues (87). There is evidence that bone marrow-derived cells normally reconstitute mesangial and interstitial cells (88, 89). Imasawa et al. (88) demonstrated that GFP⁺ bone marrow cells migrate to glomeruli and interstitium and contribute to the normal cell turnover.

Although the majority of regenerating tubular epithelial cells are derived from an intrarenal source (90, 91), bone marrow-derived cells may contribute to the replacement of tubular epithelial cells through a process of cell fusion (92, 93). The hematopoietic cell type that is responsible for the cell fusion-derived epithelial cells is unclear, although growing evidence suggests that macrophages are involved, as has been shown in the liver (93, 94). Recently, Li et al. (92) used *cre/loxP* recombination under the direction of the kidney-specific cadherin promoter with sex-mismatched bone marrow transplantation to demonstrate that fusion occurs in post-ischemic kidneys and in cocultures of bone marrow cells and renal epithelial cells *in vitro*. Macrophages demonstrate cell plasticity and have the ability to undergo cell-cell fusion with themselves or other cell types, particularly in response to inflammatory stimuli (95). Mature blood monocytes and inflammatory macrophages have been shown to transform into vascular elements including endothelial cells, myofibroblasts, and smooth muscle cells in addition to neuronal and liver cells (87, 96–98). Taken together, the discovery that cell fusion events occur between renal cells and macrophages or their highly proliferative progenitors suggests a scientific basis for new cell therapy approaches for organ regeneration.

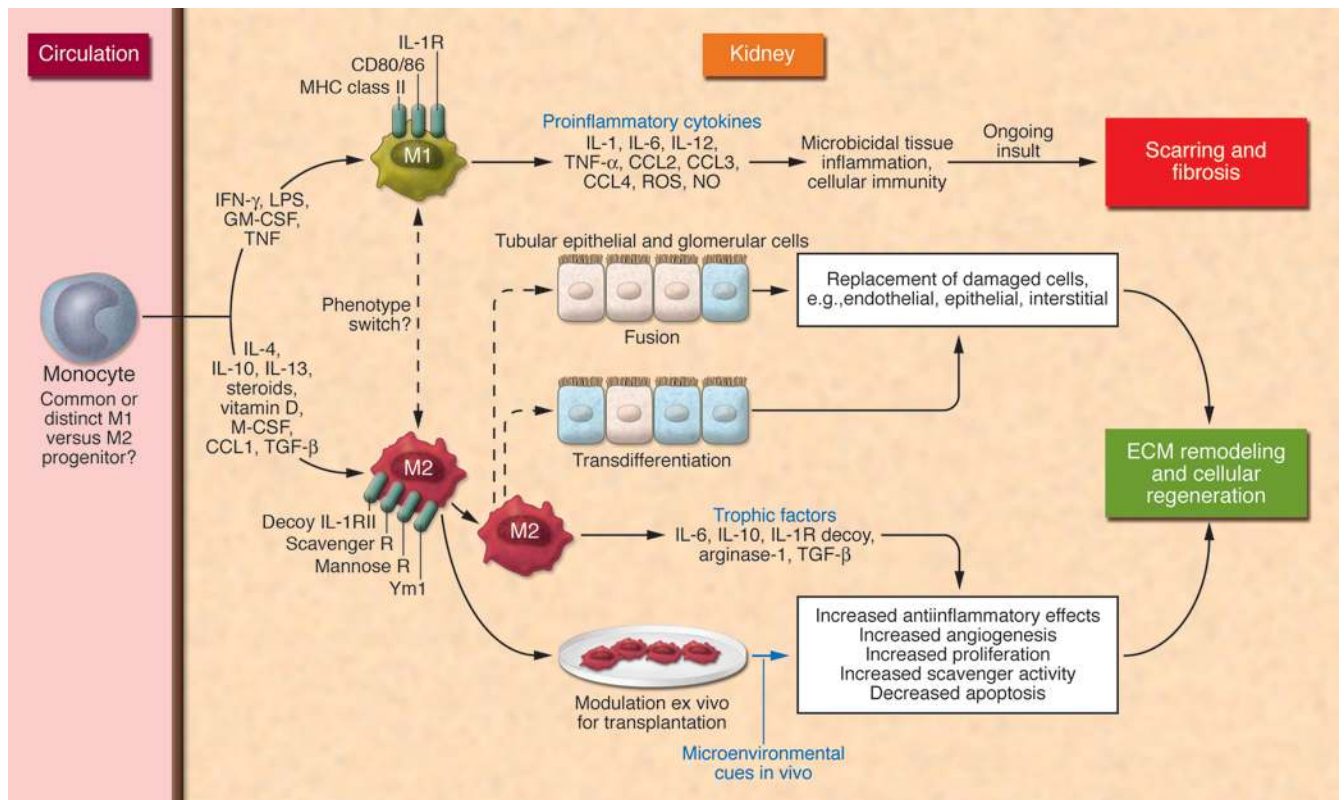


Figure 2

Macrophage phenotype and function are critical determinants of fibrotic scarring or resolution of injury. Monocytes from the circulation that enter the kidney in response to inflammatory cues undergo distinctive pathways of differentiation into classically activated M1 macrophages or the alternative M2 phenotype. Activation of M1 inflammatory macrophages by classical immune pathways may lead to the expression of MHC class II antigens and release of proinflammatory cytokines. In response to ongoing injury, M1 macrophages propagate inflammation and ultimately the development of fibrosis. Dependent on microenvironmental cues, M2 macrophages may be recruited from the circulation or activated in situ as a result of an M1-to-M2 phenotype switch. M2 antiinflammatory macrophages secrete regenerative trophic factors that promote cell proliferation and reduce apoptosis and stimulate angiogenesis. Macrophages derived from engrafting bone marrow myeloid progenitors may contribute to the repopulation of injured tubular epithelial and glomerular cells by a process of transdifferentiation or cell-cell fusion, leading to replacement of damaged cells. Ex vivo modulation of macrophages to form an M2 phenotype for transplantation may be used therapeutically to suppress the immune response and promote tissue remodeling, leading to structural repair and functional recovery. R, receptor.

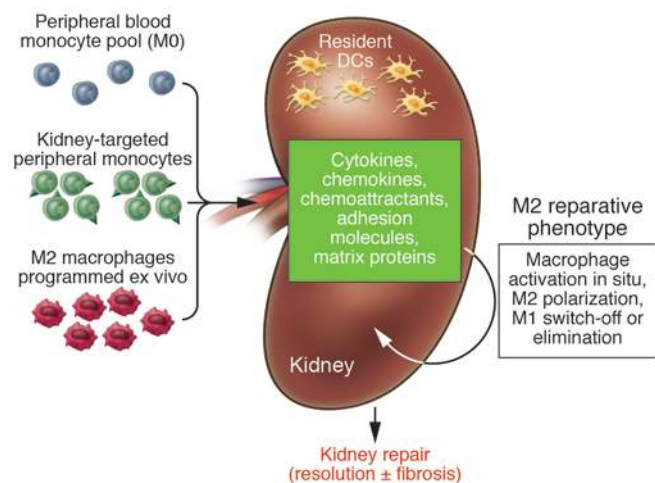
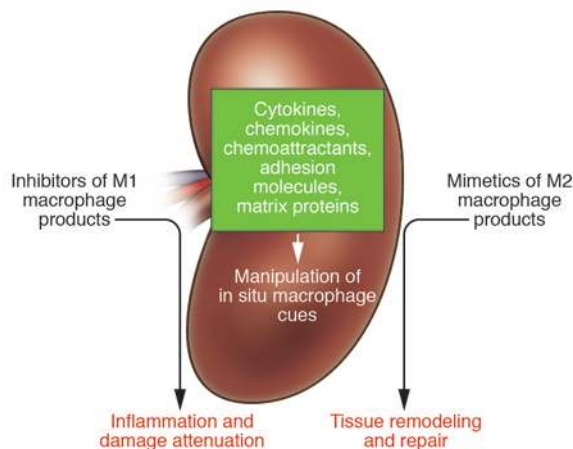
In search of the beneficial role of macrophages: implications for renal therapy

Recognition of macrophage functional diversity offers the possibility of new therapies for patients with chronic kidney disease given the nearly universal colocalization of interstitial macrophages within regions of kidney fibrosis and nephron destruction (60). However, it must be acknowledged that the M1/M2 macrophage phenotype and function paradigm is based largely on studies in mice; extrapolation to man must proceed with caution (99). An additional challenge pertains to the fact that although an M2-skewed macrophage response is associated with inflammation resolution and tissue healing, these cells also promote fibrosis. The mechanisms that fine-tune the M2-associated response to achieve repair without scarring and long-term consequences are unknown and demand attention. Assuming that these questions can be answered, it is easy to envisage novel cellular therapies based on the infusion of pre-programmed macrophages or molecular therapies that program or mimic the macrophage phenotype in vivo. The goal of discovery of effective treatment for progressive disorders such as chronic kidney disease should guide future investigations in several areas.

Engineering monocytopoiesis

It is assumed that the majority of kidney injury-associated macrophages are derived from the circulating monocyte pool, as resident kidney interstitial macrophages and dendritic cells are usually considered terminally differentiated and nonproliferating. Derived from CD34⁺ bone marrow progenitors, it is unclear whether the M1 and M2 macrophages originate from common or distinct lineages. In either case, interventions designed to block M1 generation or enhance M2 polarity may be therapeutically feasible (Figure 3).

One can envisage cellular therapies based on the infusion of peripheral monocytes primed ex vivo by exposure to a cocktail of cytokines (IL-4 and IL-13, for example) to induce M2 macrophages, as reported in a mouse model of kidney disease (73). PPAR γ exposure has been reported to skew monocytes toward an antiinflammatory phenotype (100). Ideally, such cells would be enriched for specific chemokine/chemoattractant receptors that facilitate the preferential migration of these cells to sites of kidney damage. Such an approach could be aided by the identification of new M2 phenotypic markers that also confer biological functions associated with tissue repair. As previously mentioned, the recent

**A Cell-based therapy following kidney injury****B Secretome-based therapy following kidney injury**

study in mice lacking SHIP, an endogenous inhibitor of the PI3K pathway, suggests that PI3K activation may be necessary for M2 programming and offers another potential priming strategy (51).

Tissue-specific monocyte recruitment

It is unclear whether chemokines, other chemoattractants, and/or adhesion molecules that attract monocytes to sites of injury also determine monocyte phenotype as they differentiate into macrophages, or whether secondary molecular signals are required to polarize macrophages once they are within a unique microenvironment within a damaged kidney (Figure 2A). Monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR2 are associated with an M1 response; blocking this pathway experimentally has already been shown to reduce kidney fibrosis (101–103). CCR2 is also a signaling receptor that activates NF- κ B. Perhaps this pathway can provide the secondary signal leading to M1 phenotype acquisition. Experimental manipulations that block NF- κ B have also been shown to attenuate proteinuric kidney disease (104). However, macrophage phenotype does not always correlate with function. For example, high expression of the fractalkine receptor CX3CR1 aligns with M2 polarity in atherosclerosis and in healing ischemic myocardium and cutaneous wounds, yet CX3CR1 inhibition has been reported to have beneficial effects

Figure 3

Future therapeutic possibilities for kidney disease. (A) Macrophage-based cellular therapies or therapeutic interventions can be envisaged that capitalize on the specialized macrophage secretome that determines differential function. Monocytes might be manipulated ex vivo to migrate to a damaged kidney, where they are preferentially M2 polarized, perhaps by inducing receptors to specific chemokines or chemoattractant molecules. Alternatively M2-type macrophages generated ex vivo from peripheral blood monocytes can be administered. Renal dendritic cells and unpolarized macrophages (M0) might also be skewed to an M2 phenotype by therapeutic manipulation of intrarenal molecular signals, such as specific cytokines, chemokines, or ECM proteins, known to direct this process in situ. (B) As more is learned about which soluble secreted macrophage products are associated with renal injury versus repair, single agents or, more likely, a cocktail of biological agents, drugs, and/or small molecules, might be administered to direct tissue recovery. This might include targeting of secondary intracellular signaling cascades that are activated by specific macrophage-derived products.

following renal ischemia (101, 105–107). Clearly more information about the large family of chemokine receptors may offer a unique opportunity to co-manipulate macrophage recruitment and function in pathological states.

Site-specific cues for macrophage polarization

It is conceivable that critical molecular changes that typify the renal response to injury could be exploited therapeutically. If macrophage polarization occurs locally as a secondary event after recruitment, several innovative therapies can be envisaged that block M1-polarizing pathways. The fact that the currently available TNF- α -blocking agents have been disappointing when tested as treatment for aggressive kidney diseases such as Wegener granulomatosis highlights the challenge of translating in vitro observations into complex in vivo milieu (108). This may be a situation wherein a cocktail of agents are needed to effectively “switch off” proinflammatory M1 macrophages.

An alternative approach might be to enhance endogenous pathways that defend against tissue damage by selectively modifying macrophage function. For example, activated HGF has impressive antiinflammatory and antifibrotic effects in several experimental disease models (109, 110). Enhanced local expression of HGF-activating proteases, inhibition of endogenous inhibitors of HGF activation (e.g., HGF activator inhibitor type 1 [HAI-1] and HAI-2), or administration of exogenous HGF are predicted to attenuate chronic kidney disease, but whether this is accomplished through effects on macrophage programming remains to be determined.

Interventions that re-engineer the molecular composition of wound-associated ECM can be envisaged to skew macrophage polarity. Osteopontin is an ECM protein and monocyte chemoattractant that accumulates in the interstitium in chronic kidney disease (111). An exciting observation made in a skin wounding model was that osteopontin antisense therapy not only reduced inflammation but allowed the skin to heal faster and without scars (112). Deposition of the soluble form of the small proteoglycan biglycan within the ECM can activate a proinflammatory macrophage program via TLRs (113). Renal scar tissue is not simply an inert network of fibrillar collagens, but a dynamic structure comprised of osteopontin, biglycan, and several additional components that may communicate with neighboring cells including macrophages; modification of scar tissue constituents



offers another therapeutic approach to influencing macrophage phenotype. An area deserving of further investigation is whether resident kidney interstitial macrophages and dendritic cells can be manipulated therapeutically toward a phenotype associated with wound healing (114). Another important question is whether the acquisition of M1 or M2 states represents terminal differentiation, or the phenotype is plastic and interconversion is feasible.

Macrophage receptors and soluble secreted products

The M2 macrophage phenotype is typically associated with high-level scavenger receptor expression (e.g., the classical SRA/B scavenger receptor MARCO, CD163, the mannose receptor, uPAR, and the AT1; refs. 24, 71, 115). As a group, these receptors serve a multitude of diverse functions, but they share in common the ability to mediate endocytic clearance. This activity is often associated with injury resolution, offering the possibility that macrophage manipulations to augment scavenger receptor levels might be a therapeutic goal.

Additional macrophage profiling studies based on their functional diversity may not only identify new membrane receptors that confer functional specificity but may further elucidate their unique biosynthetic profiles as therapeutic hopefuls (116). For example, it is already known that protection from ROS and NO-derived oxidant species produced by M1 macrophages offers many potential benefits for patients with chronic kidney disease, but development of more potent antioxidant drugs directed against specific targets is desirable. M2-derived products shown to promote tissue repair represent new candidates for therapeutic peptide development. For example, the IL-1 decoy receptor and MMP-13 are M2 products ascribed antiinflammatory and tissue remodeling effects, respectively (117, 118). Detailed delineation of the M2 macrophage molecular signature also offers the possibility of new insights into pathways that differentiate scarred from scarless wound healing. Arginase-1 expression associated with the M2 phenotype converts L-arginine to L-ornithine. While this reaction might be beneficial as it shunts L-arginine away from iNOS and NO-derived species generation, L-ornithine is a precursor for proline, which enhances collagen biosynthesis (119). Identifying an alternative pathway for L-ornithine consumption might reduce scarring in the presence of arginase activity. The IL-13 pathway may offer another therapeutic opportunity. While IL-13 is associated with M2 polarity, the presence of the IL-13 decoy receptor (IL-13R α_2) has been shown to decrease pulmonary fibrosis (120). Systemic *Il13* gene therapy reduces renal tubulointerstitial damage and inflammation caused by ischemia-reperfusion in rats (121). More therapeutic targets are likely to emerge as genomics, proteomics, and metabolomics further define the M2 phenotype and function.

Tissue macrophage turnover

Another aspect of macrophage biology that might be exploited therapeutically pertains to the pathway of elimination. Differentiated macrophages have a finite lifespan and presumably undergo apoptotic death; it seems unlikely that they exit from sites of injury and die elsewhere. Macrophage half-life is limited by the macrophage activation state, the inflammatory milieu, and pro-apoptotic stimuli that determine the frequency of apoptosis (122, 123). Differential gene expression studies have identified significant differences in the expression of a subset of apoptosis-related genes (116). Renoprotective interventions might include strategies that increase M2 macrophage survival relative to M1 at sites of kidney injury.

Genotype-specific therapy?

A major clinical challenge for practicing nephrologists is the lack of precise methods to predict which patients with early chronic kidney disease are at risk for progression to end-stage kidney disease. As the continuum of macrophage functional variation becomes more clearly defined, “staging” studies of injury-associated kidney macrophages present in biopsy specimens may prove to be an informative prognostic indicator. It is also plausible that genetic polymorphisms may determine activity levels for some of these key macrophage functional proteins, as shown for MCP-1, for example, and that sometime in the future peripheral blood genotyping will be useful both to identify patients at risk for progressive kidney disease and to tailor design biological therapeutics (124, 125).

In summary, cells of the myeloid lineage are hailed to the kidney in response to injury. In response to local environmental cues, they acquire specialized functions selected from a huge repertoire. Major functional categories include phagocytic scavenging, synthesis of a myriad of soluble secreted products, immune surveillance as APCs, and cellular fusion partners. In a context-dependent manner, functionally polarized intrarenal macrophages may truly serve as “friend” or “foe.” As these molecular phenotypes become further defined, new macrophage-based therapies for fibrodestructive disorders such as chronic kidney disease should emerge.

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