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Macrophage-Mediated Injury and Repair After Ischemic Kidney Injury

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Abstract

Acute ischemic kidney injury is a common complication in hospitalized patients. Currently no treatment is available for augmenting kidney repair or preventing progressive kidney fibrosis. Animal models of acute kidney injury demonstrate that activation of the innate immune system plays a major role in the systemic response to ischemia/reperfusion injury. Macrophage depletion studies suggest that macrophages, key participants in the innate immune response, augment the initial injury after reperfusion, but also promote tubular repair and contribute to long-term kidney fibrosis after ischemic injury. The distinct functional outcomes seen following macrophage depletion at different time points after ischemia/reperfusion injury suggest heterogeneity in macrophage activation states. Identifying the pathways that regulate the transitions of macrophage activation is thus critical for understanding the mechanisms that govern both macrophage-mediated injury and repair in the post-ischemic kidney. This review examines our current understanding of the complex and intricately controlled pathways that determine monocyte recruitment, macrophage activation, and macrophage effector functions after renal ischemia/reperfusion injury. Careful delineation of repair and resolution pathways could provide therapeutic targets for the development of effective treatments to offer patients with acute kidney injury.

Keywords

Macrophage activation; ischemia/reperfusion; acute kidney injury; repair

Introduction

Ischemic renal injury remains a major cause of acute kidney injury, contributing to an increased risk of in-hospital mortality as well as long-term risk of chronic kidney disease, end-stage renal disease, and mortality. Unfortunately, there is no available treatment that will either enhance kidney repair or prevent the possibility of progressive kidney fibrosis following an episode of acute kidney injury. Our understanding of the histopathologic course of ischemic kidney injury is limited as renal biopsies are seldom performed in these patients. Rodent models of ischemic kidney injury demonstrate a robust innate immune response in the kidney after ischemia/reperfusion (I/R). Early after reperfusion, the endothelium is activated, leading to increased permeability and expression of chemokines that promote

homing of leukocytes into the kidney [1]. IFN γ -producing NK T cells and neutrophils are early responders, trafficking into the kidney within hours after reperfusion and contributing to early tubular injury [2]. Monocytes infiltrate the injured kidney shortly after neutrophils at 24 hours after reperfusion, differentiate into macrophages and increase in numbers through day 7 during the renal tubular recovery phase. *In vivo* macrophage ablation studies suggest that macrophage activation after renal I/R injury is temporally and functionally dynamic, transitioning from a pro-inflammatory to a reparative phenotype. The mechanisms that regulate macrophage activation and effector functions over the course of acute ischemic kidney injury and repair are not well understood. This review will focus on the pathways that are implicated in regulating macrophage-mediated injury and repair in rodent models of renal I/R.

The Macrophage: Definitions and Origins

Over a century ago, Elie Metchnikoff first identified cells capable of nutrient and foreign body uptake in simple marine organisms [3]. He named these cells phagocytes and classified phagocytes with a simple non polymorphic nucleus as macrophages and smaller phagocytes with a large polymorphic, fragmented nucleus as microphages, now called neutrophils. Since their description, the classification and definition of macrophages have been a challenge. Recent studies with lineage tracing and ablation of bone marrow hematopoiesis in mice have redefined our understanding of macrophage lineages [4, 5]. The mononuclear phagocytic system is an ontological definition of phagocytes and defines phagocytes into at least three lineages: yolk sac, fetal liver, and bone marrow. These lineages are regulated by the colony stimulating factor-1 receptor (CSF1R) and its ligands IL-34 and CSF1. Bone marrow-derived myeloid precursors give rise to circulating monocytes. In mice, monocytes are divided into 2 subsets, defined by Ly6C and chemokine receptor expression: Ly6C⁺CCR2⁺ (pro-inflammatory) and Ly6C⁻X3CR1⁺ (resident or patrolling). Recent studies suggest that monocyte subsets differ in their expression profiles, functional roles, and trafficking behavior [6]. Ly6C⁺ monocytes give rise to F4/80^{low} macrophages and dendritic cells (DCs), the latter under the regulation of Fms-like tyrosine kinase 3 ligand (FLT3L) [5, 7].

Kidney macrophages are chimeric in origin as they derive from both the yolk sac and the bone marrow. Kawakami and colleagues recently characterized resident kidney mononuclear phagocytes and identified five distinct populations based primarily on surface expression of CD11b and CD11c, but also on surface expression of F4/80, CD103, CD14, CD16, and CD64 [8]. Recent genomic and transcriptional profiling of dendritic cells and macrophage subsets suggests that the cell surface marker CD11c may not be sufficient to distinguish macrophages and dendritic cells, as macrophages can also express CD11c [7, 9]. When citing data, this review will refer to the mononuclear phagocyte cell types as per the authors' original definition in their studies. However, interpretation of prior studies using CD11c as an inclusion or exclusion marker for intrarenal dendritic cells and macrophages, respectively, may require careful interpretation.

Macrophage functions and activation states

Metchnikoff's phagocytosis theory laid the foundation for our current understanding of innate immunity and the essential role of macrophages in host defense. Macrophages are well-known as initial responders in host defense: taking up exogenous pathogens, initiating the anti-microbial response, and instructing adaptive immunity. Beyond establishing the concept that macrophages play a major role in host defense against exogenous pathogens, Metchnikoff also postulated that phagocytes are critical components of normal development and maintenance of homeostasis in the adult organism. Indeed, recent studies have elucidated the many roles of macrophages including tissue morphogenesis, metabolism, and tissue repair [5].

The innate immune response to tissue injury involves the local production of chemokines that promote the recruitment of neutrophils and naïve monocytes to the site of inflammation where they receive local signals that regulate their phenotypic responses. For monocytes, these local signals promote maturation into phagocytic macrophages that secrete factors that can in turn augment, modulate, or suppress the local inflammatory milieu. *In vivo* studies of animal models of infectious or sterile organ injury suggest that macrophages are initially induced to a pro-inflammatory state that promotes the removal of infected or apoptotic cells, followed by a wound healing state that contributes to the process of repair. Anti-microbial and wound healing responses are self-limited once the pathogen or noxious stimulus is eliminated. However, in the setting of chronic infection or injury, persistent macrophage activation and abnormal wound healing processes can result in fibrosis.

Macrophage Activation

Due to the diversity of macrophages, attempts have been made to classify macrophages by activation and functional states. Macrophage activation can be classified into two major categories: classically activated M1 macrophages and alternatively activated M2 macrophages. These categories were initially defined by *in vitro* responses to the prototypic T helper 1 (Th1) cytokine, interferon- γ (IFN γ), or Th2 cytokines, IL-4 and IL-13, respectively [10]. The original M1/M2 designation has since been expanded to include a growing set of inducers and transcriptional regulators that mediate a wide spectrum of activation states.

Classically activated M1 macrophages

IFN γ acts through the IFN γ receptor complex (IFNGR) composed of the IFNGR1 and IFNGR2 chains on the surface of macrophages to activate JAK1/2-STAT1 dependent expression of pro-inflammatory chemokines, including macrophage chemoattractant protein 1 (MCP1, CCL2) and monokine induced by gamma interferon (MIG, CXCL9) that recruit monocytes and T cells, respectively, to sites of inflammation [11]. IFN γ also induces the expression of inducible nitric oxide synthase (iNOS), a key effector in pathogen killing through the production of nitric oxide (NO). In concert, locally released factors that are derived from either pathogens (termed pathogen-associated molecular patterns, PAMPs) or from injured host cells themselves (termed danger-associated molecular patterns, DAMPs) can signal to induce pro-inflammatory macrophage activation. PAMPs, such as

lipopolysaccharide, are recognized by pattern recognition receptors (PRR) such as toll-like receptors (TLR) [12]. Recent studies suggest that endogenous DAMPs released following sterile injury, such as high mobility group box 1 (HMGB1), ATP, extracellular matrix proteins, and nucleic acids are also recognized by PRRs [13]. Recognition of PAMPs and DAMPs by PRRs activates intracellular signaling via transcription factors such as NF- κ B and IRF5 resulting in the induction of a complementary array of pro-inflammatory responses including IL-1, IL-6, IL-12, and TNF α [14, 15]. The functional role of classically activated macrophages is host defense, mediating anti-microbial activity, and the expression of antigen presentation genes. Although they can cause tissue damage, pro-inflammatory M1 monocytes/macrophages can play an important role in clearing apoptotic cells and debris after sterile injury thereby initiating the repair response, as seen after muscle injury [16].

Alternatively activated M2 macrophages

Alternatively activated M2 macrophages have a diverse functional repertoire, contributing to wound healing, fibrosis, insulin sensitivity, and immunosuppression. M2 macrophages are sub-categorized into wound healing and immunoregulatory macrophages, although there is likely to be considerable overlap. Wound healing M2a macrophages are typically defined by IL-4/IL-13 stimulated induction of factors such as arginase-1 (ARG1), mannose receptor (MR), insulin like growth factor (IGF1), FIZZ1, and Ym1 [17]. The canonical M2a signaling pathway is mediated through IL-4 and/or IL-13 binding to the IL-4R α receptor, which in turn activates the JAK-STAT6 signaling pathway [18]. STAT6 is a transcription factor that can induce M2 activation by regulating the transcription of M2 effectors (ARG1 and Ym1, e.g.) often in association with additional transcriptional regulators such as peroxisome proliferator-activated receptor- γ (PPAR γ), Kruppel-like factor 4 (KLF4) and C/EBP β [19–22]. The transcription factor IRF4 can mediate alternative activation both downstream and independent of STAT6 activation [23, 24]. Functionally, IL-4/IL-13 activation of macrophages leads to upregulation of growth factors such as IGF1, and stimulation of arginase activity, leading to the conversion of L-arginine to L-ornithine and urea. Ornithine can then be metabolized into polyamines, which are important for cell proliferation, as well as proline, the precursor to collagen synthesis and thus extracellular matrix production [25]. This increase in the production of growth factors and extracellular matrix components is important in normal wound healing, but can promote fibrosis if sustained or abnormally regulated.

Immunoregulatory macrophages are induced by TLR ligands, immune complexes, glucocorticoids, IL-10, prostaglandins, and phagocytosis of apoptotic cells. Immunoregulatory macrophages produce high levels of IL-10, an immunosuppressive cytokine. Thus, it is proposed that the primary function of these regulatory macrophages is to limit inflammation through dampening the immune response. In addition to the production of IL-10 [26], phagocytosis of apoptotic cells also leads to macrophage production of transforming growth factor- β (TGF- β) [27], which acts to inhibit pro-inflammatory cytokine production, but is also considered to be potently pro-fibrotic. Some have proposed to further sub-categorize immunoregulatory macrophages to M2b (induced by TLR ligands and immune complexes) and M2c (induced by IL-10 and/or TGF- β) [28].

Macrophage plasticity and in vivo activation

M1 and M2 macrophages have largely been defined through *in vitro* stimulation experiments. *In vivo* studies of macrophages suggest a much more complex phenotype that is determined by tissue-specific differentiation signals and the microenvironment, consisting of numerous activating stimuli that vary not only among disease models but also temporally with respect to different stages of injury and repair [29]. The temporally dynamic continuum of macrophage phenotypes could result from either ongoing recruitment of new monocyte populations that are activated by a changing tissue microenvironment, differential activation of distinct subsets of myeloid cells within the kidney after injury, or plasticity in macrophage activation states. These hypotheses to explain phenotypic divergence of mononuclear phagocytic cells, recently reviewed in detail [30], are all likely to contribute to the diversity of tissue macrophages.

Pro-inflammatory Macrophages Contribute to Ischemic Renal Injury

As noted above, the macrophages that accumulate immediately after renal I/R injury express high levels of pro-inflammatory mediators including IL-1, IL-12, and iNOS. The recruitment of these macrophages to the injured kidney involves the release of chemokines such as MCP1 and fractalkine that act via surface receptors on circulating monocytes. CCR2 is a major chemokine receptor that regulates the MCP1 dependent recruitment of pro-inflammatory monocytes mediating early tubular injury after renal I/R. Studies using the CCR2 knockout mouse model have shown that recruitment of circulating CCR2⁺ monocytes into the kidney 24 hours following I/R injury plays a critical role in promoting tubular injury [31, 32]. The functional role of the fractalkine receptor CX3CR1 in macrophage recruitment and renal I/R injury is less clear. *Cx3cr1^{GFP/GFP}* mice, in which GFP has been knocked-in to the *Cx3cr1* locus and CX3CR1 is not expressed, demonstrated functional renal protection 24 hours after I/R [31], whereas there was no difference in tubular necrosis at 24 hours after I/R in *Cx3cr1^{-/-}* mice [33]. Blockade of other chemokine receptors, such as CCR1 and CCR5, does not prevent the pro-inflammatory macrophage phenotype during the early stages after renal I/R. Blockade or deficiency of CCR1 results in fewer macrophage in the injured kidney, but fails to alter the degree of renal injury [34].

CCR5 mediates renal injury after I/R via T cell activation, but does not affect macrophage infiltration [35]. Studies utilizing liposomal clodronate, an encapsulated bisphosphonate that induces apoptosis upon phagocytic uptake, suggest that depletion of pro-inflammatory M1 macrophages at the time of renal I/R injury is protective, while adoptive transfer of *in vitro* M1 activated macrophages promotes injury [36–41]. In contrast, macrophage depletion with diphtheria toxin (DT) in the CD11b-DTR mouse model at the time of renal I/R injury is not protective. Studies comparing the two models of macrophage depletion suggest that the protective effect of liposomal clodronate during the early injury phase may be due to its effect on reducing the pro-inflammatory cytokine profile [41] and the persistence of an intrarenal CD206 positive macrophage population that is resistant to clodronate depletion but sensitive to DT-mediated ablation [40]. The different functional outcomes of macrophage depletion during the early phase of renal I/R injury with the two ablation

models further implicate the likelihood of multiple subsets of kidney macrophages in the overall post-ischemic immune response.

The molecular mechanisms by which pro-inflammatory macrophages promote injury within the kidney early after reperfusion are not well understood. The early reperfusion period during which tubular injury occurs is dominated by neutrophil degranulation, reactive oxygen species production, release of DAMPs from necrotic and injured tubular cells, as well as release of IFN γ by Th1 T cells and NK T cells [42]. This microenvironment is poised to induce infiltrating monocytes to differentiate into M1 pro-inflammatory macrophages through the activation of STAT1 and TLR/NF- κ B pathways by IFN γ and DAMPs, respectively. CD11c⁺ DCs contribute to the pro-inflammatory environment through sphingosine 1-phosphate receptor-3 (S1P3) dependent Th1 activation of IFN γ -producing NK T cells [43]. IFN γ -dependent induction of iNOS in M1 pro-inflammatory macrophages leads to the production of NO, which upon interaction with reactive oxygen species, will generate cytotoxic peroxynitrites [44]. The role of myeloid TLR/NF- κ B activation by DAMPs is unclear. Studies of bone marrow chimeras established with wild-type mice and *Tlr2*^{-/-} or *Tlr4*^{-/-} mice would suggest that epithelial TLR signaling plays the dominant role in mediating tubular damage, rather than TLR signaling from infiltrating bone marrow derived cells [45, 46].

Some negative regulatory pathways that limit the pro-inflammatory activation of resident DCs after renal I/R have been identified. The Single Ig IL-1-related Receptor (SIGIRR), a negative regulator of TLR/IL-1 receptor signaling, limits the level of intrarenal dendritic cell pro-inflammatory activation after renal I/R injury [47]. Activation of the adenosine A2 receptor (A_{2A}R) suppresses dendritic cell mediated Th1 activation of NK T cells [48]. Targeting the SIGIRR and A_{2A}R receptors could help limit inflammation and prevent tubular injury after renal I/R.

Macrophage-Mediated Repair After Renal I/R

In contrast to the protective nature of macrophage depletion at the time of renal I/R, macrophage depletion during the tubular repair phase delays normal tubular proliferation. Macrophage ablation by either liposomal clodronate in wild-type mice or DT in the CD11b-DTR mouse model 48–72 hours after I/R inhibits tubular repair [38, 49, 50]. The mechanisms by which kidney macrophage activation changes from pro-inflammatory to reparative are not fully defined in the post-ischemic kidney. While distinct macrophage populations can be recruited and/or activated during the different phases of injury and repair, the anti-inflammatory effect of phagocytosis and the milieu of the microenvironment are likely to predominate in the induction of reparative and anti-inflammatory macrophages.

It is well characterized that phagocytosis of apoptotic cells can alter macrophage expression to promote anti-inflammatory macrophage activation with increased IL-10 and TGF- β production [26, 27]. Both ischemically injured tubular cells and infiltrating neutrophils undergo apoptosis in the 24–72 hours after I/R injury, and thus, the phagocytosis of these cells is likely to contribute to the phenotypic switch from pro-inflammatory to anti-inflammatory macrophage activation. The microenvironment also plays a significant role in

instructing macrophage activation within the kidney. While adoptive transfer of resting RAW 264.7 macrophages reconstitutes injury at 24 hours after I/R in macrophage-depleted mice, adoptive transfer of resting macrophages during the repair phase, 72 hours after I/R, promoted kidney repair [39]. Our laboratory has demonstrated that IFN γ -stimulated, M1 pro-inflammatory bone marrow-derived macrophages (BMM) injected into a mouse during the injury phase retain their M1 phenotype [38]. Whereas, M1 BMM injected during the reparative phase, 3 days after renal I/R injury, switch towards an alternative activation phenotype (loss of iNOS and upregulation of MR expression) within the kidney. These data suggest that macrophages alter their expression profile in response to temporally dynamic signals from the local kidney milieu. *In vitro* co-culture studies suggest that tubular cells secrete factors that can induce macrophage alternative activation [38]. CSF1 has been implicated as a potential kidney-derived signal that can mediate macrophage alternative activation. Exogenous CSF1 can promote an M2 phenotype in kidney macrophages and renal repair after renal I/R injury [51]. However, in experiments comparing blockade with a CSF1 receptor specific antibody and macrophage depletion using DT in the CD11b-DTR model, macrophages only partially contributed to CSF1-dependent tubular repair after I/R injury [52]. Identification of kidney-derived signals that mediate macrophage alternative activation will advance our understanding of how the microenvironment within the post-ischemic kidney can induce the reparative macrophage phenotype.

One approach in defining these kidney-derived signals is via identification of the transcriptional regulation of the alternative activation of kidney macrophages. Studies in knockout mice deficient for the main components of the canonical M2 alternative activation pathway, IL-4 and STAT6, suggest a significant protective role of IL-4 and STAT6 signaling in the functional response to renal I/R injury, although macrophage phenotypes were not reported in that study [53]. *In vitro* BMM and proximal tubular co-culture studies show that secreted tubular factors can induce macrophage alternative activation independent of IL-4R α and STAT6 signaling [38, 54]. Data from *Il4ra*^{-/-} mice subjected to renal I/R injury are consistent with this, showing that IL-4R α signaling is not required for normal tubular repair or macrophage alternative activation [38]. Macrophage activation and expression phenotypes will have to be directly compared in these whole body *Il4*^{-/-}, *Stat6*^{-/-}, and *Il4ra*^{-/-} knockout models in order to determine their impact on macrophage activation and reconcile the functional differences observed following renal I/R injury.

IRF4, a transcription factor that mediates M2 macrophage alternative activation in adipose tissue macrophages and in response to helminthic infections [24, 55], may play a role in macrophage alternative activation in the reparative response to renal I/R injury. Lassen and colleagues showed that IRF4 is upregulated in resident CD11c⁺ dendritic cells after renal I/R and that *Irf4*^{-/-} mice exhibit increased tubular injury and whole kidney pro-inflammatory cytokine expression after I/R [56]. One can postulate that the lack of IRF4 resulted in the failure of kidney macrophage alternative activation, however M2 markers were not reported in their study. Recent characterization of renal I/R injury in *p53*^{-/-} mice suggests that p53 may also regulate macrophage activation [57]. Whole body p53 knockout mice exhibited worse tubular injury and impaired tubular repair, both correlating with a persistence of pro-inflammatory macrophages, decreased KLF4 expression, and failure to induce alternative activation in kidney macrophages. Bone marrow chimeras with wild-type and *p53*^{-/-} mice

suggest that the absence of p53 expression in bone marrow-derived cells was sufficient to explain the phenotype of the whole body p53 knockout. It remains to be determined if p53 suppresses pro-inflammatory macrophage activation or directly regulates alternative activation gene expression.

Macrophage-Derived Reporative Effector Molecules

Although ARG1, IGF1, and mannose receptor are widely used to identify M2 macrophages, the specific functional roles of these macrophage-derived factors or receptors in promoting repair after renal I/R injury have not been determined. ARG1 is upregulated in kidney macrophages during the tubular repair phase after I/R [38]. One can postulate that the functional significance of macrophage ARG1 in promoting tubular repair is due to its enzymatic production of polyamines and proline to promote cell proliferation and collagen synthesis, respectively. However, arginase activity can have multiple effector functions. Arginase also competes with iNOS for L-arginine thereby limiting NO production. Myeloid expression of ARG1 in parasitic infections suppresses CD4 T cell proliferation and activation, highlighting a potential role of ARG1 in suppressing Th2 dependent inflammation and fibrosis [58]. IGF1 is a growth factor known to promote tubule cell proliferation and kidney repair in rodent models of I/R injury [59]. Thus, the local secretion by alternatively activated macrophages may also play a significant role in promoting tubule repair.

In contrast to these well-described M2 factors, several additional proteins expressed by alternatively activated macrophages have been investigated in kidney injury models. Macrophage-secreted chitinase-like protein BRP-39 is critical for normal tubular repair after I/R injury, and acts by limiting tubular apoptosis through activation of tubular cell PI3K/Akt signaling [60]. I/R injury resulted in increased tubular injury and mortality in *Brp39*^{-/-} mice compared to wild-type mice. Macrophage-derived Wnt7b also plays an important role in stimulating repair and regeneration of renal epithelial cells after ischemic injury by limiting tubular apoptosis and cell cycle arrest [50]. Finally, the downregulation of macrophage hemoxygenase-1 (HO-1) in aging mice has been shown to result in an increased susceptibility to renal I/R injury [61]. Clearly, more studies are required in order for us to fully understand the specific M2-dependent pathways that promote tubular repair.

Macrophage Cell-Based Therapy

Studies using cell therapy with transduced or *ex vivo* stimulated macrophages to express a reparative or anti-inflammatory phenotype have highlighted inducible macrophage specific pathways that can limit tubular injury and promote renal repair. When injected at the time of I/R injury, HO-1 overexpressing macrophages trafficked to the post-ischemic kidney and helped preserve glomerular filtration [62]. The mechanism of macrophage-derived HO-1 renal protection is postulated to be due to enhanced phagocytosis of apoptotic cells and reduction in microvascular platelet deposition. Similarly, IL-10 over-expressing macrophages, when administered at the time of I/R injury, were able to preserve renal function, limit tubular injury and reduce pro-inflammatory cytokine production within the kidney [63]. These IL-10 over-expressing macrophages exert their protective effect through the production of lipocalin-2, which protects against tubular apoptosis and promotes tubular

proliferation in an iron-dependent pathway that leads to upregulation of HO-1 [64]. Recently, Ranganathan and colleagues showed that adoptive transfer of netrin-1 treated macrophages also mitigates early tubular injury after renal I/R through a PPAR γ dependent pathway [65].

Role of Macrophages in Post-Ischemic Kidney Fibrosis

While the presence of macrophages is critical for normal tubular repair after ischemic injury, the persistence of an activated macrophage infiltrate correlates with progressive tubulointerstitial fibrosis and impaired renal function [66]. Blockade or deficiency of CX3CR1 provides a modest reduction in the development in the interstitial fibrosis after renal I/R injury [33], whereas, long-term macrophage depletion via liposomal clodronate treatment initiated 72 hours after I/R injury more clearly attenuates the development of late renal fibrosis [67]. The protective effect of macrophage depletion and inhibition of monocyte recruitment suggests that macrophages contribute to the development of fibrosis. While macrophage infiltration is often used as an endpoint measure of the severity of fibrosis, the mechanisms by which macrophages persist after the acute repair phase and promote fibrosis remain unclear.

Macrophages are known to secrete many factors associated with fibroblast activation and the progression of fibrosis, such as TGF- β 1, platelet-derived growth factor, fibroblast growth factor 2, insulin-like growth factor binding protein 5, and galectin-3 [68]. TGF- β 1 has long been implicated as central player in the development and progression of fibrosis [69]. As macrophages are known to produce significant amounts of TGF- β 1, especially in the setting of phagocytosis of apoptotic cells, we had hypothesized that macrophage-derived TGF- β 1 would be a significant contributor to the development of fibrosis after renal I/R injury. Although we found that *Tgfb1* expression is highly upregulated in renal macrophages following kidney injury, myeloid-specific deletion of *Tgfb1* did not significantly attenuate progression of renal fibrosis after ischemic injury despite significant reductions in whole kidney *Tgfb1* expression and downstream Smad signaling [70]. Recent work by Campanholle and colleagues suggests that myeloid-specific signaling downstream of MyD88, an adaptor protein for all TLRs except TLR3, also does not contribute to the development of renal fibrosis after I/R injury [71].

Although the effector pathways that induce the pro-fibrotic actions of macrophages in the kidney after I/R injury remain elusive, mechanisms of suppressing inflammation and inhibiting macrophage dependent fibrosis are emerging. Pentraxin-2 (also known as serum amyloid P) is a circulating serum protein that can activate macrophages through binding Fc γ receptors and inducing IL-10 expression, leading to an immunoregulatory macrophage phenotype capable of preventing the progression of fibrosis in both unilateral ureteral obstruction and I/R renal fibrosis models [72].

Conclusion

The timing of macrophage activation states following kidney injury is critically important in determining the balance between initial tubular damage, subsequent repair and late fibrosis.

Induction of an M2 wound healing activation state is required for tubule cell proliferation and repair, but must be followed by a transition to an immunosuppressive resolution phase in order to avoid the development of progressive chronic fibrosis. Interest is growing in defining ways to suppress M1 macrophage activation and promote M2 wound healing macrophage activation at the time of injury in order to decrease tubule injury and enhance repair, followed by immunoregulatory macrophage activation to suppress inflammation and activate collagen remodeling to prevent late fibrosis [68]. Current data on the role of macrophage activation in ischemic kidney injury have all derived from animal models of renal I/R injury. As sequential kidney biopsies are rarely performed in patients with acute tubular necrosis, these findings have not yet been confirmed in humans. If data from animal models are found to correlate with biopsy findings in patients who suffer from ischemic kidney injury, then understanding the mechanisms that control macrophage activation and effector functions will lead us towards potential macrophage-targeted therapies for ischemic kidney injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Macrophage Activation and Effector Responses after Renal I/R

Early after renal I/R injury, PMNs and NK T cells are recruited to the kidney. ROS and IFN γ released by activated PMNs and NK T cells as well as tubule cell-derived DAMPs promote M1 activation of infiltrating monocytes. M1 macrophages in turn upregulate iNOS leading to the production of nitric oxide that can promote local formation of peroxynitrites and induction of tubular cell apoptosis. S1P3 signaling on resident DCs promotes Th1 activation of NK T cells. SIGIRR and A_{2A}R on DCs provide negative feedback to limit the level of pro-inflammatory activation. During the proliferative tubular repair phase, macrophage derived BRP-39 and Wnt7b promote tubular regeneration by limiting tubular apoptosis. Serum amyloid P (pentraxin-2) induces immunoregulatory macrophages that express IL-10 and promote resolution of the inflammatory process thereby limiting the development of fibrosis. The mechanism by which macrophages promote renal fibrosis after I/R injury is unclear.

A_{2A}R, adenosine A2 receptor receptor; DAMP, danger associated molecular pattern; DC, dendritic cells; I/R, ischemia/reperfusion; IFN γ , interferon- γ ; M Φ , macrophage; M1, M1 macrophage; M2a, M2 wound healing macrophage; ROS, reactive oxygen species; PMN, polymorphonuclear neutrophils; NK, natural killer T cells; NO, nitric oxide, S1P3, Sphingosine 1-phosphate receptor-3; SIGIRR, Single Ig IL-1-related receptor.

Table 1

Targeting Macrophage Recruitment and Activation After Renal ischemia/reperfusion (I/R).

Experimental Method		Outcome	Ref
Macrophage Depletion			
Prior to I/R	Liposomal clodronate 1–4 days prior to renal I/R injury. Outcomes 24 hours after I/R	Decreased histologic and functional tubular injury. Decreased whole kidney expression of pro-inflammatory cytokines	[36–41]
	Diphtheria toxin 18–24 hours prior to renal I/R in CD11b-DTR mice. Outcomes 24 hours after I/R	No protection in histologic or functional renal injury. No difference in whole kidney expression of pro-inflammatory cytokines	[40, 41]
During Repair	Liposomal clodronate Initiated 48 hours after I/R Initiated 6 days after I/R	Decreased improvement in renal function and tubular proliferation day 5 and 7 after I/R. Increased tubular apoptosis on day 8.	[38] [49]
	Diphtheria toxin in CD11b-DTR Initiated 3 days after I/R	Decreased improvement in renal function and persistence of tubular injury day 6 after I/R	[50]
Prolonged depletion	Liposomal clodronate administration starting at 72 hours after I/R, then every 5 days.	Decreased interstitial collagen deposition and whole kidney pro-inflammatory cytokine and TGF- β 1 expression at 4–8 weeks after I/R	[67]
Blocking Monocyte Recruitment			
	<i>Ccr1</i> ^{−/−} CCR1 antagonist	Decreased macrophage infiltration. Not renoprotective at early or late time points (1–7 days after I/R).	[34]
	<i>Ccr2</i> ^{−/−} Inhibition of CCR2/MCP1 signaling	Decreased monocyte recruitment. Decreased histologic and functional renal injury at 24 hour after I/R.	[31, 32]
	<i>Cx3cr1</i> ^{GFP/GFP} <i>Cx3cr1</i> ^{−/−}	Preserved glomerular filtration rate (GFR) at 24 hours after I/R. No difference in tubular necrosis at 24 hours, modest decrease in fibrosis at 7 and 14 days after I/R.	[31] [33]
Targeting Myeloid Polarization			
M1	<i>S1pr3</i> ^{−/−}	Chimeric mice suggest that S1P3 signaling on bone marrow-derived cells mediate renal I/R injury 24 hours after I/R. Adoptive transfer of WT but not S1P3-deficient DCs activated NK T cells.	[43]
	<i>Tlr2</i> ^{−/−} <i>Tlr4</i> ^{−/−}	Chimeric mice suggest that renal parenchymal TLR signaling rather than TLR activation in bone marrow derived cells mediate renal I/R injury.	[45, 46]
	<i>Sigirr</i> ^{−/−}	Increased histologic and functional renal injury. Chimeric mice suggest that SIGIRR signaling in bone marrow derived myeloid cells limits tubular injury by suppressing activation of intrarenal myeloid cells.	[47]
	<i>Adora2a</i> ^{−/−} <i>Cd11c-Cre;Adora2a</i> ^{fl/fl}	Increased histologic and functional renal injury. Adoptive transfer of WT DCs treated with A _{2A} R agonists decreased histologic and functional renal injury by suppressing NK T cell production of IFN γ	[48]
M2	<i>Il4ra</i> ^{−/−}	No difference in functional renal injury or repair. No difference in the induction of MR expression in kidney macrophages (day 7 after I/R). <i>Arg1</i> and <i>MR</i> mRNA expression are induced in both wild-type and <i>Il4ra</i> ^{−/−} macrophages co-cultured with tubular cells.	[38]
	<i>Il4</i> ^{−/−} and <i>Stat6</i> ^{−/−}	Increased histologic and functional renal injury. M2 markers not assessed.	[53]

Experimental Method		Outcome	Ref
	<i>Irf4</i> ^{-/-}	Increased histologic and functional renal injury. M2 markers not assessed.	[56]
	<i>p53</i> ^{-/-}	Increased histologic and functional renal injury. Increased number of macrophages and fewer MR+ macrophages 7 days after I/R.	[57]
	Exogenous administration of CSF1	At 5 and 7 days after I/R: Decreased histologic and functional tubular injury Increased expression of M2 markers in kidney macrophages.	[51]
	Depletion of macrophages with DT in CD11b-DTR mice compared to CSF1R neutralizing antibody	Blockade of CSF1R hindered normal tubular repair more than ablation of macrophages alone.	[52]
Targeting Effector Molecules and Signaling Pathways			
Injury/Repair	Hemoxygenase (HO-1) induction with heme arginate	Loss of macrophage expression of HO-1 in aging mice mediates increased renal injury 24 hours after I/R. Macrophage depletion with DT in CD11b-DTR mice reversed tubular protection by heme arginate in aging mice.	[61]
	<i>Brp39</i> ^{-/-}	Decreased survival after I/R. Increased histologic and functional renal injury at day 3 after I/R. Macrophage BRP-39 limits tubular apoptosis through a PI3K/Akt dependent pathway.	[60]
	<i>Csf1r-iCre;Wnt7b</i> ^{C3/-}	Increased histologic and functional renal injury at day 7 after I/R. Macrophage Wnt7b promotes tubule basement membrane regeneration and overcomes G2 arrest in kidney epithelial cells after I/R.	[50]
Inducible pathways to limit injury	Adoptive transfer of HO-1 overexpressing BMM	Decreased microvascular platelet deposition and preserved GFR 24 hours after I/R.	[62]
	Adoptive transfer of IL-10 overexpressing BMM	Decreased histologic and functional tubular injury 24 hours after I/R via an iron dependent lipocalin-2 pathway.	[63]
	Adoptive transfer of netrin-1 treated BMM	Decreased functional and histologic renal injury 24 hours after I/R. Promoted M2 polarization through a PPAR γ dependent pathway.	[65]
Fibrosis	Exogenous recombinant human serum amyloid P (pentraxin-2)	Inhibited kidney fibrosis after I/R, acting through activating Fc γ receptors on macrophages and production of IL-10.	[72]
	<i>LysM-Cre;Tgfb1</i> ^{fⁿ}	Decreased whole kidney <i>Tgfb1</i> expression and downstream signaling with minimal effect on the development of renal fibrosis after I/R.	[70]
	<i>LysM-Cre;Tgfb1</i> ^{fⁿ}	No effect on the development of renal fibrosis after I/R.	[71]