Macrophages in Kidney Injury and Repair

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Macrophages: Tissue Effector Cells of the Monocyte Lineage

Macrophages (Mφs) are monocyte-derived tissue effector cells from hematopoietic stem cells in bone marrow via circulation. Their expansion in tissues can either be due to local proliferation of resident cells or infiltration from the circulation, depending on the stimulus (1-3). Beyond their traditional role in protecting the host from invading pathogens, Mφs have been shown to play expansive roles as regulators of development, tissue homeostasis, remodeling, and repair (4). Although the key role of Mφs in kidney repair after injury is gaining considerable appreciation, along with neutrophils, Mφs have a reputation of being leukocytes which drive tissue injury and fibrosis in immunological and chronic inflammatory kidney diseases (5-8). During disease, Mφs expand even in the absence of pathogens, and disperse after repair or recovery (8). However, a large population of Mφs persists after expansion in chronic progressive kidney disease (3, 9). In many human kidney biopsy studies, Mφs have been found in large numbers in both acute diseases such as post-streptococcal glomerulonephritis (GN), anti-neutrophil cytoplasmic antibody (ANCA) associated GN, or in chronic diseases such as IgA nephropathy or lupus nephritis, and also in acute and chronic kidney transplant disease (10-17). Many studies have shown that the tissue of Mφs are in fact not merely passive bystander cells, but are activated and involved in the pathogenesis of kidney diseases. These observations hold true not only in immunological kidney diseases such as GN but also in non-immunological diseases, such as diabetic nephropathy, ischemic/vascular kidney disease, and all forms of chronic kidney disease (18-27). In all of these diseases, the cell numbers and activation status of Mφs have been reported to correlate positively with disease severities (28-32).

Activation of Macrophages

Activation of Mφs is a key element of initiating an efficient immune response or participating the tissue repair and regeneration. A wide range of stimuli such as conserved microbial structures, so-called pathogen-associated molecular patterns (PAMPs), have been shown to activate Mφs through a limited number of pattern recognition receptors (PRRs) which include toll-like receptors (33-36). These PAMPs include natural ligands on microbes, such as lipopolysaccharide (LPS), peptidoglycans as well as nonessential proteins such as flagellin. In addition to the natural ligands, several PRRs interact with endogenous, host-derived ligands including modified protein, carbohydrate, lipid, nucleic acids, nuclear proteins and mitochondrial proteins broadly known as dangers associated molecular patterns (DAMPs) (37-42). Immune complexes (ICs) (comprising immunoglobulins, antigens, complement components, pentraxins and other plasma proteins of the innate immune system) frequently deposit in the glomerulus and ligate activating immunoglobulin Fc receptors (FcRs) to complement receptors (CRs); they also have the capacity to activate Mφs with broadly similar activation and pattern of cytokine release (43). Release of DAMPs may be central to regulating the responses of Mφs to tissue injury. By activating intracellular signaling pathways that include nuclear factor κB and mitogen-activated protein kinase, Mφs spew out a broad range of pro-inflammatory cytokines including tumor necrosis factor α (TNFα), interleukin (IL)-1β, IL-6, IL-12, IL-18, IL-23 and pro-inflammatory chemokines including macrophage inflammatory protein (MIP), monocyte chemotactic protein and chemokine (C-X-C motif) ligand 1 (CXCL1, 45-57, 2012
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neutrophil chemoattractant). Mφs can also generate reactive nitrogen and oxygen species, including nitric oxide. This pattern of activation is broadly known as classical or M1 activation and can be detected by cell surface upregulation of TREM-1, Ly6C, FcγRI or expression of inducible nitric oxide synthase (iNOS) or IL-1β proteins (44, 45). By contrast, certain pathogens, such as amoebae and schistosomes, activate Mφs in a different manner. In response to these pathogens, Mφs generate high levels of transforming growth factor β (TGFβ), IL-10, IL-13 and chemokines including chemokine (C-C motif) ligand 17 (CCL17) and CCL22. This alternative pattern of activation is known as M2 activation and has been associated in vivo with wound healing. In the presence of a cell surface Ed2 antigen (CD163) in rats or the cell surface markers galectin 3 (Mac2), scavenger receptors including the mannose receptor, MARCO, and the transferrin receptor in mice have been implicated as markers of M2 type (wound healing) Mφs in tissues. Certain DAMPs, such as adenosine, have now been reported to drive Mφ activation towards an M2 phenotype and such polarized Mφs promote wound-healing functions. However, most DAMPs broadly identified activate Mφs in a M1-type activation and it is likely that additional signals at the Mφ cell surface are important in determining cell phenotype. Importantly, it is worth stating that these imposed classifications of Mφ activation states are an oversimplification and may not hold up across different organs and disease settings. In any event, the capacity of Mφs to engage in and perform injurious or wound-healing functions probably depends on specific and efficient activation.

In addition to the production of cytokines and chemokines, a major role for Mφs is their capacity for phagocytosis (46). In addition to invading microbes, Mφs scavenge aged erythrocytes, dying leukocytes, cellular debris, pathological matrix, and ICs (47). In all these circumstances, phagocytic clearance can occur without cellular activation and in the context of kidney disease would be beneficial to tissue remodeling and regeneration following injury (48-51). In fact, when healthy, most monocytes do not become significantly activated but rather clear things away (erythrocytes, neutrophils, ICs), thus, it is likely that the major normal function of monocytes/Mφs is a wound-healing non-inflammatory role, and it is only under overwhelming circumstances that cellular activation occurs and pro-injurious factors are liberated. In this context it is very striking that in models of single kidney injury and repair such as the ischemia reperfusion model (a model of human acute tubular necrosis (ATN)), inflammatory Mφs are recruited during the repair phase and these Mφs correlate numerically with repair (7, 8). One collective interpretation of these observations is that the physiological role of Mφs is one of an attempt to initially sterilize and debride a tissue, followed by repair of the tissue (52, 53). However, in repetitive or chronic injury states, the Mφs are driven to become excessively or aberrantly activated with deleterious effects.

**How Macrophages Activated in Injured Kidney?**

Since Mφs are present in diverse kidney diseases, there has been debate about how Mφs are activated (Fig. 1) (3, 54-57). Several studies in mouse models of GN (nephrotoxic nephritis (NTN) and the anti-glomerular basement membrane (anti-GBM) disease) have indicated that Mφs are secondary effectors regulated by CD4+ T lymphocytes reactive against foreign antibodies planted in the glomerulus (54). It is likely that this type of T lymphocyte-directed activation of Mφs takes place through paracrine signaling by interferon γ (IFNγ), IL-12, IL-17, and other Th1- or Th17-skewed lymphokines. While this mechanism of Mφ activation is likely important in certain contexts in human glomerular diseases, it is unlikely that this is a major mechanism of Mφ activation in the kidney because there is relatively little evidence of cell-mediated autoimmunity against the kidney itself in diseases such as ANCA associated GN, and lupus nephritis. The exception to this is anti-GBM and Goodpasture’s syndrome (58-60). Many ‘immunological diseases’ of the kidney feature deposition of ICs in the glomerulus. In these diseases, the glomerulus in large part is a bystander, rather than a target, of autoimmunity. ICs become trapped in the glomerulus by virtue of its highly specialized vasculature and sieving function. It is likely that the major physiological function of neutrophils and monocytes/Mφs in the glomerulus is safe, ‘non-phlogistic’, phagocytic clearance of formed ICs from the glomerulus (61, 62). This innate immune function is complex in that it involves many innate immune proteins, receptors and regulated cell signaling pathways, with many systems in place to prevent myeloid leukocyte activation triggered by this cleaning-up process, including complement proteins and pentraxins (47, 63). However this ‘non-phlogistic’ clearance of ICs is readily overwhelmed with resultant consumption of plasma complement, inappropriate myeloid leukocyte activation, and liberation of local cytotoxic products and pro-inflammatory chemokines which contribute to local tissue injury, activation of the coagulation cascade, and recruitment of further leukocytes with consequent loss of glomerular function (9, 54, 64-66). The consequence of this is that the net effect of the monocyte/
Mφ function is deleterious in several models of GN (9, 66, 67). The activating Fcγ receptors (and FcεR) and late complement components (including C5a) have been strongly implicated in this activation process (61, 68-72). In addition to IC GN, increasing understanding of the mechanisms of ANCA associated GN presenting as rapidly progressive GN also suggest that local activation of neutrophils and monocytes/Mφs in glomerular capillaries by ANCA ICs on the endothelial surface is an important part of the pathogenesis of glomerular injury in these diseases (73). Therefore similar arguments for the factors and mechanisms involved in activation of leukocytes in IC diseases such as lupus nephritis, probably hold true for ANCA associated GN too (9, 61, 67).

In addition to the immunological kidney diseases many other kidney diseases feature the involvement of glomerular and interstitial Mφs. Diabetic nephropathy, chronic kidney disease of any initial etiology, acute interstitial nephritis (AIN), and ATN all feature marked recruitment of interstitial Mφs (47). While it has been easier to understand how glomerular Mφs clearing ICs or those adjacent to activated T lymphocytes might become activated, it is less clear how interstitial or glomerular Mφs in non-immunological kidney disease become activated. One possibility is that natural killer (NK) cells, recruited to sites of injury also liberate IFNγ (Fig. 1). Another possibility is that chemokines such as CXCL1 released from injured parenchymal cells not only recruit Mφs but also activate them (74). However, in many diseases of the kidney there are very few NK cells and data showing that chemokine ligation of monocyte chemokine receptors triggers activation has not been forthcoming (54). It is more likely that factors released from injured parenchymal cells or extracellular factors oxidized or modified during injury function as ligands for activating receptors on Mφs (Fig. 1) (75). These DAMPs have been increasingly described as binding to receptors of the innate immune response known as PRRs triggering leukocyte activation, which has a similar pattern of activation as PAMPs and ICs. This group of danger molecules includes advanced glycation endproducts, ribonucleoproteins containing CpG sequences, high mobility group box 1, adenosine, histone 4, mitochondrial DNA, and others (37-42).

**Heterogeneity of Macrophages**

As an increasing number of cell-surface Mφ markers have been identified using antibodies; labeling studies have shown different populations of Mφs in the kidney and elsewhere (3, 5, 8, 9, 29, 47, 76-89). These Mφ subpopulations have been found to produce different cytokines and behave differently. In vitro studies indicate that Mφs can be polarized to phenotype M1 or M2 after activation by different cytokines. However in vitro studies are highly artificial and may not reflect the scenario in vivo. Not only are the cytokines IFNγ, LPS, IL-4, IL-13 which generate different subpopulations of Mφs less abundant in an injured kidney, but the correlation between in vitro markers and in vivo function is also poor (3, 47). Hence the classification of Mφ subpopulations has been modified to reflect the controversy (Fig. 1). The M1 or classical activated population of Mφs can generate a broad range of pro-inflammatory cytokines, chemokines, and reactive nitrogen and oxygen species, including nitric oxide. This pattern of activation is broadly known as classical or M1 activation. The M2 population of Mφs may be better described as wound-healing since depending on the injury and organ context the M2 Mφs may promote wound healing, angiogenesis, or fibrosis. In addition, exposure to apoptotic cells, the anti-inflammatory cytokine IL-10 or the ICs can generate Mφs that produce high levels of IL-10 themselves and are actively involved in the suppression of immune responses. This Mφ subpopulation might be better identified as a regulatory Mφ (Mreg) (47, 90, 91). This Mreg macrophage is defined by high levels of IL10 production. IL10 may be an effector of function in certain settings but in many settings IL10 is merely a marker of regulatory function. Other genes including CXCL9, -10, and -11 may play roles in the Mreg phenotype.

Three hypotheses explaining heterogeneity of monocytes/Mφs have been proposed: (1) monocytes differentiate into infinite subpopulations depending on the environment (Hume Hypothesis) (92); (2) pre-existing subpopulations of monocytes are functionally prescribed (Geissmann/Randolph Hypotheses) (93-95); (3) discrete functional subpopulations can switch from one to the other (52). Evidence supports all three of these hypotheses. In vitro cultured Mφs acquire different phenotypes transcriptionally depending on the cytokine mixture applied, suggesting infinite possibilities. Furthermore, in vivo studies demonstrate that monocytes not only sense danger or injury but also respond to the tissue specific environment and acquire multiple phenotypes (3, 96-98). In contrast, studies from the 1990’s revealed two or more discrete subpopulations of human monocytes and studies by Geissmann and Randolph et al. provided evidence of clear functional differences between subpopulations of circulating monocytes in mice, in keeping with the second hypothesis (93, 99-101). Our own studies using the marker Ly6C to define Mφ subpopulations confirm the second hypothesis but show that the third hypothesis holds true in vivo. That is, a single monocyte subset differentiates sequentially into functionally discrete populations rather than infinite phenotypes.
Fig. 1. Subpopulations of macrophages *in vivo*. Schematic showing three different types of macrophages, and factors that regulate their activation/differentiation in sterile inflammation *in vivo*. Although local factors can regulate recruited monocytes to differentiate into different macrophage subpopulations, regulatory macrophages also differentiate from classically activated (M1) and wound-healing (M2) macrophages, triggered by mechanisms that are poorly understood (3, 47).

Fig. 2. Model showing Ly6C<sup>high</sup> monocytes are preferentially recruited to the injured kidney compared with Ly6C<sup>low</sup> monocytes from capillary, which differentiate into three populations of kidney macrophages, Ly6C<sup>high</sup>, Ly6C<sup>int</sup> and Ly6C<sup>low</sup>. A pool of mature Ly6C<sup>high</sup> monocytes is released from bone marrow in response to kidney injury. These kidney macrophage subpopulations generate discrete M1-biased (Ly6C<sup>high</sup>) and M2-biased (Ly6C<sup>low</sup>) cytokines *in vivo*. The Ly6C<sup>int</sup> subpopulation comprises both macrophages derived from activation of resident macrophages and also macrophages in transition with Ly6C<sup>high</sup> and Ly6C<sup>low</sup> subpopulations (3).
Local release of adenosine in injured tissues binds adenosine receptors on \( \text{M} \)\( \phi \)s and can trigger polarization of \( \text{M} \)\( \phi \)s (3, 74). Further studies to define the role of locally released adenosine, DAMPs, and their cognate receptors in this switch are required. It would also be interesting to study the role of the cross talk between the influxed monocytes and local cells in determining the phenotypic switch (102-104).

**Lessons from Genetic Models in Rodents**

Until recently, the function of \( \text{M} \)\( \phi \)s in tissue injury has largely been inferred by their presence in injured tissues and the cytokines that they can generate *in vitro* when activated. A limited number of studies have use polyclonal anti-\( \text{M} \)\( \phi \) sera suggested deleterious functions for \( \text{M} \)\( \phi \)s in glomerular diseases, but these have to be interpreted with caution due to lack of specificity of such preparations (66, 85). Liposomal encapsulated clodronate has been developed recently to ablate \( \text{M} \)\( \phi \)s *in vivo*. This strategy relies on the selective uptake of liposomes by monocytes and \( \text{M} \)\( \phi \)s, delivering toxic levels of the bisphosphonate clodronate. However, liposomes are endocytosed by many cells including neutrophils and endothelial cells, and clodronate has anti-inflammatory effects.

*Genetic Models for Macrophage Ablation in Mice*

We developed a genetic approach to ablate \( \text{M} \)\( \phi \)s *in vivo*, relying on the selective susceptibility of human cells but not mouse cells to the toxic effects of diphtheria toxin (DT) (9, 105). Humans are more than 1000 fold more susceptible to DT than rodents due to the cell surface expression of the human heparin binding epithelial growth factor receptor which is a receptor for DT (DTR) and transports DT to the cytosol where it is extremely lethal. We generated a mouse model *CD11b-DTR* where the DTR was expressed under a \( \text{M} \)\( \phi \)-specific promoter for the integrin CD11b. Although CD11b is expressed by other cells including neutrophils, only monocytes, \( \text{M} \)\( \phi \)s, dendritic cells, and NK cells are susceptible to DT (6). Using this model we have been able to target monocytes and \( \text{M} \)\( \phi \)s specifically in models of kidney disease, at different time-points. In a model of crescentic GN induced by nephrotoxic serum, \( \text{M} \)\( \phi \) ablation prevents disease progression including interstitial fibrosis and tubular atrophy. In a second model of cyroglobulinemia-associated membranoproliferative GN, the overall effect of \( \text{M} \)\( \phi \) ablation was amelioration of disease (62). Hence, \( \text{M} \)\( \phi \)s are deleterious in these models of glomerular disease.

In order to explore the role of \( \text{M} \)\( \phi \)s in fibrosis progression further in `non-immunological` disease of the kidney, we used a simple model of mechanical injury caused by obstruction of the ureter of the kidney that results in inflammation and fibrosis (3, 47). We discovered that \( \text{M} \)\( \phi \)s also promote fibrosis in response to mechanical injury, indicating the generalized role for \( \text{M} \)\( \phi \)s in fibrosis progression but also indicating that much of the interstitial disease seen in immunological kidney disease may be in response to secondary cellular injury rather than glomerular ICs.

A major function for \( \text{M} \)\( \phi \)s is phagocytosis of dying cells and debris and this may be considered a component of the repair process. Furthermore, \( \text{M} \)\( \phi \)s may produce cytokines including IL-10, hepatocyte growth factor, vascular endothelial cell growth factor, and WNT ligands, which may drive both repair and regeneration of parenchymal cells, and also limit activation of leukocytes. In a model of kidney injury followed by repair, the ischemia reperfusion injury model (IRI), \( \text{M} \)\( \phi \) recruitment coincides with repair not injury (7, 8). In the *CD11b-DTR* mouse model, ablation of \( \text{M} \)\( \phi \)s during the repair phase after IRI does indeed prevent normal repair (8), and these findings have been confirmed by ablation sutides using liposomal clodronate. Studies are currently underway to dissect the mechanisms by which \( \text{M} \)\( \phi \)s promote repair in single injury followed by repair and regeneration whereas they promote cell loss and fibrosis in repetitive injury or chronic injury.

**Rodent Congenics**

Strains of inbred mice and rats have widely differing susceptibility to kidney diseases. These differences have been exploited to identify the genes that determine disease susceptibility (106, 107). The Wistar Kyoto rat strain is extremely susceptible to the model of IC GN induced by nephrotoxic serum (NTN). This type of rat was crossed with Lewis rats which are resistant to developing disease. By back-crossing and testing for disease susceptibility, two novel disease susceptibility genes have been identified and they are both monocyte/\( \text{M} \)\( \phi \) genes. One Fc\( \gamma \) receptor 3 (Fc\( \gamma \)RIII) is present in an alternate form in susceptible rats. This renders its \( \text{M} \)\( \phi \)s unable to efficiently phagocytose ICs, and renders them more active by pathways other than Fc\( \gamma \)RIII (61). The other disease susceptibility gene is JunD, a transcription factor in the activator protein-1 family that regulates activation of \( \text{M} \)\( \phi \)s (67). These studies serve to both highlight the central role of \( \text{M} \)\( \phi \)s in nonphlogistic clearance of ICs.
and intracellular regulation of Mφ activation as key facets that regulate disease progression. Furthermore, rodent FcγRIII is analogous to human FcγRIIA, a major activating FcγR. This receptor has many polymorphisms that determine susceptibility to the development of systemic lupus erythematosis (SLE) and lupus nephritis (108, 109). In congenic studies in mice that develop spontaneous SLE, several disease susceptibility chromosomal loci have been identified. The mouse Sle1 locus is syntenic with the human SLE susceptibility loci containing genes involved in the complement cascade and FcγRs, which are present on monocytes and Mφs. It also contains genes for B-cell survival signals, regulatory T cells and complement receptor 2, and regulates the development of autoantibody and nephritis (106, 110-112). These studies therefore also place monocytes and Mφs at the center of the immune response in these models of lupus nephritis. Collectively, these powerful genetic studies place the regulation of Mφ activation and signaling through Mφ FcγRs at the center of the immune response in the glomerulus.

**Mechanisms of Macrophage-Mediated Fibrosis**

In recent studies, we have identified a primary role of Mφs in kidney fibrosis. Together with reports of similar roles in other organs, this pro-fibrotic role of Mφs is suggested to be a stereotyped response to chronic injury or repetitive injury (3, 9, 105, 113-120). Multiple mechanisms by which Mφs cause fibrosis have been proposed in different disease contexts (98, 121, 122). Arginase generated by Mφs may directly promote fibrosis by hydrolyzing arginine to ornithine which can be used to generate polyamines directly promote fibrosis by hydrolyzing arginine to ornithine that may be playing a role in fibrogenesis, it is not likely to be the major Mφ effector cytokine.

Fibrocytes, it has been suggested that myeloid cells, which express CD34 and high levels of class II MHC and also generate collagenous matrix directly, play a role in fibrosis of many organs (130-133). Despite reports providing evidence for the presence of fibrocytes in models of kidney disease, our recent exhaustive studies of these cells indicates that in mice, at least, collagen producing myeloid leukocytes are rare in kidney disease and do not contribute directly to fibrogenesis (134). It is more likely that fibrocytes serve as antigen presenting cells and recruit more monocytes/Mφs which directly or indirectly signal myofibroblasts and their precursor’s pericytes via cellular (paracrine) cross talk (134).

To understand the mechanisms of Mφ-mediated fibrogenesis further, we have recently defined subpopulations of Mφs in the kidney by the cell surface marker Ly6C (Fig. 2) (3, 47). In chronic kidney disease models including the ureteral obstruction model, three populations of kidney Mφs can be identified (Ly6C<sup>high</sup>, Ly6C<sup>int</sup>, Ly6C<sup>low</sup>). All three may derive from a single population of circulating inflammatory Ly6C<sup>high</sup> monocytes. Ly6C<sup>high</sup> Mφs in the kidney are activated, producing pro-inflammatory cytokines including IL-1β and chemokines including MIP1α, MIP2. These Mφs are similar to M1 activated Mφs, but occur in sterile injuries without ICs, implicating the role of DAMPs in the process of Mφ activation. In stark contrast, although Ly6C<sup>low</sup> Mφs derive from Ly6C<sup>high</sup> Mφs, they generate low levels of IL-1β and MIP2, but instead produce M2 type cytokines CCL17, CCL22, platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), angiopoietin 2 (Ang2), TGFβ and IGFBP5, all of which have been associated with fibrogenesis. Myofibroblasts have receptors for PDGF, IGF-1 and type 2 chemokines including CCR1 and CCR5. While it is possible that one Mφ-derived factor is responsible for the pro-fibrotic biology of Mφs, far more likely is that many cytokines converge on myofibroblasts or pericytes to drive fibrosis. Nevertheless, the Ly6C<sup>low</sup> Mφs, by virtue of their M2-skewed transcriptional profile, fulfill several of the criteria for paracrine signaling, and are therefore a target for therapy. Several other mechanisms by which Mφs cause fibrosis potentially exist. Firstly, Ly6C<sup>high</sup> Mφs produce M1 type cytokines including TNFα that may directly signal to myofibroblasts; secondly, if Ly6C<sup>high</sup> Mφs were prevented from becoming activated then it is likely that Ly6C<sup>low</sup> Mφs would not be activated since the latter derive from the former. Therefore, targeting activation of Ly6C<sup>high</sup> Mφs may also have therapeutic
gains. Thirdly, we have recently discovered that myofibroblasts derive from pericytes, a newly described cell type in the kidney (134, 135). Pericytes are perivascular cells of capillaries, derived from metanephric mesenchyme during development, and are necessary for angiogenesis and vascular stability through two-way signaling between pericytes and endothelial cells (136). It is therefore possible that Mφ-signaling to endothelial cells, directs pericyte migration, and differentiation into myofibroblasts. In that context, Ly6Chigh Mφs generate high levels of Ang2 which may signal deleteriously to endothelial cells with the consequence of triggering fibrosis through signaling from the endothelium.

**Mechanisms of Macrophage-Mediated Cellular Loss**

Our studies and those of others have additionally shown that in chronic or repetitive kidney diseases, Mφs not only promote fibrosis but also promote loss of epithelial cells and microvasculature (9, 129, 137, 138). The loss of epithelial cells has been detected by the presence of increased cellular apoptosis, but this cell death occurs at the same time as the proportion of epithelial cells engaged in the cell cycle increases. Thus, in these diseases, Mφs drive both the cell cycle and apoptotic cell death (9). The mechanisms by which this occurs have not been completely elucidated. Possible roles for iNOS and TNFα have been explored, and M1-skewed Mφs are implicated in this process, but no consistent cytokine signals have been clearly defined (139). Regardless of the specific cellular cross talk, two important facets of M6 biology are highlighted. Firstly, Mφ activation is required for epithelial cell death and secondly, a common theme emerges by which Mφs provoke cells into the cell cycle and target their untimely death at DNA cell cycle checkpoints. It is possible that Mφs function in this context to test cell health by triggering epithelial cells into the cell cycle. Cells will pause at a DNA checkpoint due to stress, inadequate energy or resources, or excessive damage to DNA, and are more susceptible to apoptotic cell death. Therefore, Mφs function as policemen checking for health and driving rapid death and clearance if the stress testing does not go well (4). Although this kind of function might appear desirable, excessive cell loss can ensue leading to tubule atrophy and peritubular capillary rarefaction in chronic inflammation with persistent activation of Mφs. New studies are required to understand the molecular mechanisms underlying these observations.

**Macrophages in Repair and Regeneration**

A common theme throughout this article is that the natural state of Mφs is non-phlogistic clearance of the unwanted from the body, and liberation of safe helpful cytokines that promote well-being. Chronic, repetitive or severe injury overcomes the inbuilt mechanisms that prevent activation and then Mφs become chronically activated leading to deleterious consequences. With this in mind, one might expect that Mφs play an important role in tissue repair and recovery. Indeed it is clear that Mφs are important in non-inflammatory regeneration, angiogenesis and also repair (7, 8, 140, 141). We recently found that after single injury to the kidney followed by repair there is intense recruitment of Mφs during the repair phase (8). Ablation of Mφs in this repair phase hinders tubule regeneration. At the current time, the factors that dictate how Mφs become predominantly reparative versus deleterious remain obscure. Our own studies have identified the Wnt signaling pathway as an important target of Mφs in driving regeneration (8), but doubtless multiple mechanisms have been uncovered. Consistent with previous findings, a recent study also shows that iNOS+ pro-injurious (M1) Mφs are recruited into the kidney in the first 48 hours after IRI, whereas arginase- and mannose receptor-positive, wound-healing (M2) Mφs predominate at later time points (7). In vitro studies show that IFNγ-stimulated, pro-injurious Mφs begin to express markers of M2 Mφs when cocultured with renal tubular epithelial cells. Moreover, IL-4-stimulated, M2 skewed Mφs, but not IFNγ-stimulated M1-skewed Mφs, promote proliferation of renal tubular epithelial cells *in vitro* and *in vivo*. Thus, during the normal repair of kidney injured by ischemia, Mφs acquire regenerative functions to stimulate successful epithelial proliferation.

**Monocytes and Macrophages as Targets for Kidney Disease Therapy**

**Targeting Macrophage Activation**

Inflammatory cytokines such as TNFα or IL-1β alone, or pro-inflammatory chemokines do not activate Mφs, although the lymphokine IFNγ has recognized capacity to weakly activate Mφs. Increasing evidence suggests that soluble factors and debris released from injured tissues, known as DAMPs, bind to PRRs of Mφs including toll-like receptors and other receptors such as receptors for advanced glycation endproduct (37, 41). Selective blocking myeloid cell activation triggered by DAMPs while permitting activation triggered by foreign pathogens is a highly attractive approach to the treatment of chronic inflammation since it does not pose the risk of increased infection susceptibility.

We have recently discovered that a circulating pentameric protein of the innate immune system,
serum amyloid P (SAP), which is also known as pentraxin-2 (PTX2), with structural similarities to C-reactive protein is strongly anti-fibrotic in kidney disease (47). PTX-2 deposits in injured tissues and opsonizes dead cells and debris. Once it has opsonized targets, it undergoes a conformational change converting to a high affinity ligand for the activating FcγRs, hFcγRIIA and hFcγRIII (mFcγRIII and mFcγRIV). Unlike cross-linking of FcγRs by immunoglobulin, cross-linking of FcγRs by PTX-2 does not activate Mφs. Conversely it inhibits activation mediated by other stimuli. Part of the mechanism by which PTX-2 inhibits Mφ activation is through local release of IL-10. IL-10 is an anti-inflammatory cytokine that is well recognized for inhibiting inflammation and has direct anti-fibrotic effects on myofibroblasts. By endocytosing and phagocytosing SAP-opsonized debris, Mφs release IL-10 locally in the injured kidney resulting in less activated Mφs (both M1 and M2 subtypes) that are unable to drive fibrosis. Importantly, SAP does not inhibit activation triggered by the bacterial cell wall lipoprotein LPS, implicating SAP as a novel and safe endogenous inhibitor of sterile inflammation and fibrosis. Recombinant PTX-2 is currently in Phase 2 trials as an anti-fibrotic therapy in lung fibrosis, and it clearly may have broad therapeutic indications (91). Although still in their infancy, new PRRs and DAMPs that activate the innate immune system are being identified and may become new targets for therapy (38).

Targeting Monocytes

Rodent studies using liposomal clodronate or the CD11b-DTR ablation system indicate that monocyte/Mφ ablation is effective in limiting tissue injury and fibrosis. Selective cellular ablation is widely accepted in humans by monoclonal or polyclonal antibodies, such as anti-thymocyte globulin (ATG), anti-CD3 antibodies (OKT3), anti-CD20 antibodies (rituxumab), or anti-CD52 (Campath) antibodies, which target T cells or B cells. All of these therapies are highly efficacious but come with considerable risk of infection (except anti-CD20 antibodies). ATG likely also ablates monocytes in addition to T cells. In addition, a wide range of other therapies, including mycophenolic acid, cyclosporine A, and cyclophosphamide, function either to ablate or profoundly inhibit proliferation of lymphocytes. It is quite likely therefore that monocyte specific ablative therapies would be successful in humans, but there may be unacceptable side effects, particularly if they are considered in the treatment of chronic diseases. Nevertheless, these therapies could find a role in the management of acute inflammatory diseases of the kidney including AIN and rapidly progressive GN.

The monocyte and Mφ receptor for colony stimulating factor-1 (CSF1) or the monocyte colony stimulating factor (M-CSF) is the CSF1 receptor. This tyrosine kinase dependent receptor drives monocyte proliferation in tissues and may be an alternative target for therapy. Several tyrosine kinase inhibitors that are selective for the CSF1 receptor have been developed and are in early trial phases (142).

Targeting Macrophage Recruitment

Chemokines and their receptors are important in monocyte recruitment. One problem encountered with targeting monocyte recruitment is the redundancy of chemokines and their receptors (143). Moreover, chemokine receptors such as CCR2 hold promise as new therapies in fibrosing inflammatory diseases, not only in the kidney but also in other organs (145-149). Since the innate cellular immune response to pathogens is important in health, and since almost all rodent studies are performed nowadays in sterile facilities, safety and efficacy studies will need to be completed before these compounds can be used in human diseases. In this context, the CCR2 antagonist CP-481715 (Pfizer, New York, NY) which block both CCR2 and CCR5 in humans and rodents, or combination inhibitors that block individual receptors including CCR1, CCR2 and CCR5, hold promise as new therapies in fibrosing inflammatory diseases, not only in the kidney but also in other organs (145-149). Since the innate cellular immune response to pathogens is important in health, and therefore their blockades may pose additive risks of infection. In addition, the finding that two or more subpopulations of monocytes exist in the circulation, one with a high CCR2 receptor, another with a high CCR5, hold promise as new therapies in ameliorating CCR2-dependent responses to infection overall (101). New small molecules that provide broader blockades of chemokine receptors, including compounds such as BMS-813160 (Bristol-Myers Squibb, New York, NY) which block both CCR2 and CCR5 in humans and rodents, or combination inhibitors that block individual receptors including CCR1, CCR2 and CCR5, hold promise as new therapies in fibrosing inflammatory diseases, not only in the kidney but also in other organs (145-149). Since the innate cellular immune response to pathogens is important in health, and therefore their blockades may pose additive risks of infection. In addition, the finding that two or more subpopulations of monocytes exist in the circulation, one with a high CCR2 receptor, another with a high CCR5, hold promise as new therapies in ameliorating CCR2-dependent responses to inflammation overall (101).

Targeting Macrophage Differentiation

Several studies including our own work support the model in which Mφs differentiate into a wound-
healing or pro-fibrotic Mφs (3, 37, 52, 90, 152). The mechanisms by which this differentiation occurs remain incompletely understood. Several candidate factors driving such differentiation have been described. One of which is local release of adenosine, binding to adenosine receptors. Adenosine has been ascribed as ‘anti-inflammatory’ since it can result in wound healing Mφs and prevent pro-inflammatory cytokine production. However, the role of adenosine in generating pro-fibrotic Mφs has yet to be explored. Mechanisms to selectively target adenosine receptors or target the extracellular enzymes such as CD73 which generate extracellular adenosine should be tested in models of sterile inflammation with fibrosis to determine efficacy (153).

**Other Potential Targets**

The list of Mφ paracrine effector molecules is extensive, but the precise roles these factors play individually or in concert have been inadequately tested. In Mφ cytokines PDGF, IGF-1, and Ang2, prostaglandin E2 are all cytokines liberated by M2 Mφs in vivo that may be playing deleterious roles. Our study has shown that blocking PDGF receptor signaling using monoclonal antibody or imatinib may attenuate kidney fibrosis through inhibiting pericyte-myofibroblast transition in mice with progressive kidney fibrosis (154). However, promising studies that target the PDGF receptor β, using tyrosine kinase inhibitors are currently underway in humans to determine whether selective blockades of this paracrine pathway will impact human fibrosis progression.

**Therapeutic Options in the Treatment of Chronic Kidney Disease and Immune-mediated Kidney Diseases**

In summary, Mφs are widespread in kidney diseases. Increasing evidence has shown that targeting Mφs and their functions will improve outcome in many kidney diseases. The final common pathway of chronic kidney disease that leads to organ failure increasingly appears to be driven, at least in part, by chronically activated Mφs. New therapeutics targeting Mφs are emerging and are undergoing investigation in human trials as potential new therapies in a range of kidney diseases.

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