Macrophages in Renal Disease

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ABSTRACT

Macrophages have heterogeneous phenotypes as they exercise their twofold role in the development and recovery of renal diseases. Some subpopulations of macrophages (M1) have a pathogenic function in renal inflammation, making them a logical target for elimination. Alternatively, M2 macrophage subpopulations resolve inflammation and repair injury, making them a potential therapeutic tool against renal injury. Here, we summarize recent findings regarding macrophage plasticity, and the various strategies for targeting or utilizing macrophages to treat renal disease. We highlight, in particular, the potential of renoprotective M2 macrophages to resolve inflammation and repair the kidney.


Macrophage infiltration is a common feature of most human chronic kidney disease. A critical question is whether these macrophages cause or exacerbate renal injury. Correlations between the degree of macrophage infiltration and severity of renal injury in humans, and in temporal studies in rodents, suggest an effector function for macrophages. This correlation in humans with chronic kidney disease is more of a generality but is well studied in specific diseases including IgA nephropathy and lupus nephritis. However, such strong correlations do not assure the link is causal. Proof of causality comes from animal studies that demonstrate induction of injury by pathogenic mediators of macrophages, improvement of renal injury and function by depletion of macrophages, and acceleration of renal injury by repletion of macrophages.

Studies over many years demonstrate that macrophages express many cytotoxic moieties, including proteolytic enzymes, reactive oxygen and nitrogen species, and proinflammatory cytokines and chemokines. For example, macrophages isolated from nephritic glomeruli of rats and rabbits generated reactive oxygen species and nitric oxide. Glomerular macrophages produce TNFα and IL-1β in animal models of both lupus nephritis and nephrotoxic serum nephritis. Immunohistologic studies demonstrate that macrophages infiltrating the kidneys of patients and rodents with focal necrotizing glomerulonephritis express MHC class II molecules, inducible nitric oxide synthase, and inflammatory cytokines. Activated macrophages can also induce epithelial-mesenchymal transition (EMT) forming fibroblasts by secreting matrix metalloproteinase.

A variety of strategies to deplete macrophages also prove that they contribute to renal injury. Macrophage depletion using irradiation, antimacrophage sera, lipoprotein-encapsulated drugs, or blockade of macrophage chemokine receptors reduces proteinuria and structural injury in models including antiluminal basement membrane (GBM) disease, puromycin aminonucleoside nephrosis, and Heymann nephritis.

Another powerful approach to clarify the role of macrophages in renal disease has been the use of adoptive transfer techniques. For example, transferred macrophages induce proteinuria and mesangial cell proliferation in anti-GBM nephritis. Transfer of macrophages activated by lipopolysaccharide, but not resting macrophages, also exacerbates renal injury in adriamycin nephrosis. In the latter studies, transferred macrophages accumulate progressively in sites of injury and as few as 10,000 macrophages per mouse are sufficient to exacerbate disease. These data suggest specific targeting of activated macrophages is likely to be a more effective therapeutic approach than inhibition of all macrophage subpopulations.

MACROPHAGES AS A TARGET IN RENAL DISEASE

Numerous studies over the past decade provide compelling evidence that macrophages cause tissue injury. Since the classic depletion studies of macrophages in renal disease by Holdsworth et al. in the 1980s, a variety of strategies including disruption of macrophage recruitment into kidney and genetic modification of macrophage activity by gene
intervention have been employed to reduce macrophage infiltration.

**Macrophage Depletion**

Pathogenic macrophages can be depleted directly with antisense macrophage-specific oligonucleotide in experimental glomerulonephritis, and partially reduced in adriamycin nephropathy using ED7, an antibody directed against CD11b/CD18 integrin, which is expressed by macrophages. Blockade of colony-stimulating factor receptor on macrophages, c-fms, also reduces macrophage accumulation in unilateral ureteral obstruction and murine diabetic nephropathy by markedly reducing macrophage proliferation. Treatment with liposomal clodronate also reduces the accumulation of F4/80+ macrophages and renal fibrosis in obstruction. Targeting monocyte CD11b with diphtheria toxin receptor (DTR) in a transgenic mouse (CD11b-DTR) also effectively halts progression of crescentic glomerulonephritis.

**Disruption of Macrophage Recruitment**

Inhibition of CX3CR1, the receptor for the chemokine fractalkine (CX3CL1), markedly reduces macrophage infiltration and injury in a model of crescentic glomerulonephritis. Anti-CC chemokine ligand (CCL) 2 antibody reduces macrophage infiltration, crescent formation, and proteinuria in rats with nephrotoxic serum nephritis or anti-thy 1.1 nephritis. In adriamycin nephropathy, macrophage recruitment and functional and structural renal injury are reduced by combined CCL2 and CCL5 DNA vaccination or CCL2 DNA vaccine modified to increase its immunogenicity.

However, strategies targeting chemokines or adhesion molecules have not been uniformly effective. Treatment with anti-CCL2 antibody in Wistar Kyoto (WKY) rats with anti-GBM disease did not reduce crescent formation and proteinuria, despite early reduction of macrophage infiltration. Blockade of CCL5 reduces renal injury in anti-thy 1.1 nephritis in rats and in murine nephrotoxic nephritis, but exacerbates inflammation in murine immune-complex nephritis, despite a reduction in the number of infiltrating macrophages. Moreover, antibody blockade of intercellular adhesion molecule-1 (ICAM-1) counter-receptor reduces macrophage infiltration in WKY rats with nephrotoxic nephritis; however, administration of anti-CD18 antibody against counter-receptor to ICAM-1 and -2 does not reduce macrophage infiltration in Lewis rats with nephrotoxic nephritis (Figure 1 and Table 1A).

**Genetic Alteration of Macrophage Activity**

Inhibition or enhancement of macrophage gene expression can be achieved by gene silencing or transfer using antisense oligonucleotides (ODN) to silence specific macrophage genes. In rats with nephrotoxic serum nephritis, intravenous ODN targeting NF-κB reduces macrophage infiltration, proteinuria, and proinflammatory cytokine expression. Blockade of ICAM-1 with ODN also reduces macrophage infiltration and attenuates reperfusion injury in the rat. However, inhibition of CCL2 using anti-CCL2 spiegelmers reduces macrophage infiltration, but does not improve renal pathology in mice with Alport nephropathy.

Specific genes have also been transferred to alter macrophage activation or inhibit signaling pathways. Genetically engineered macrophages can deliver genes specifically to sites of inflammation by following natural chemotactic signals. For example, macrophages transduced with adenovirus expressing IL-1ra reduce renal injury in both obstructive nephropathy and nephrotoxic nephritis. Rees and co-workers showed that macrophages transduced with IL-4 or IL-10 were able to reduce histologic injury and albuminuria in rats with nephrotoxic nephritis, but IL-10 expressing macrophages were more effective than those expressing IL-4. The same group transferred inhibitor of protein κB (1xB) into macrophages to block NF-κB, thereby reducing renal injury in rats with nephrotoxic nephritis (Figure 2 and Table 1B).

In summary, depletion of macrophages or disruption of macrophage recruitment reduces renal macrophage accumulation and injury in diseased kidney. Absence of specific surface markers for macrophages has meant that macrophage depletion strategies lack specificity. The conflicting results of blockade of macrophage chemokines or adhesion molecules suggest the pivotal importance of specific macrophage infiltration pathways and states of activation as well as the exact nature of the renal injury. Whereas the specific inhibition of macrophage genes through antisense or decoy ODN has proven successful, their utility is limited by inhibitory effects, which are short-lived (minutes to days), and by off-target activity induced by these strategies.

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**Figure 1.** Strategies for targeting macrophages to treat kidney disease. Renal injury may be reduced by strategies aimed at reducing the number and activity of effector macrophages.
reactions with other genes with sequence similarity.

Macrophages can be genetically engineered to deliver anti-inflammatory genes or inhibit signaling pathways, providing long-lasting expression and site-specific migration after chemotactic signals. However, substantial hurdles to the future clinical application of genetic transfer using macrophages include unregulated gene expression and concerns about the unproven safety of viral vectors.

**DIVERSE ROLES OF MACROPHAGES**

Macrophages comprise a heterogeneous population of cells, with diverse functions and phenotypic plasticity. Although commonly recognized for their pathogenic role in renal inflammation and fibrosis, macrophages also play critical roles in wound healing, tissue remodeling and repair, and in immune regulation. Macrophages that secrete anti-inflammatory cytokines and promote wound healing and tissue remodeling have been referred to as alternatively activated macrophages (M2). By their secretion of trophic factors and anti-inflammatory cytokines, M2 macrophages resolve inflammation and reduce injury.48,49 Macrophages are also able to fuse with themselves and other cell types, thereby providing the potential to regenerate specialized cells of the kidney.50

Three subsets of alternatively activated macrophages have been proposed:

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AN, adriamycin nephropathy; DN, diabetic nephropathy; UUO, unilateral ureteral obstruction; WKY, Wistar-Kyoto.

**Figure 2.** Mechanisms underlying beneficial effects of macrophages modified by exposure to cytokines ex vivo, or by transfection with anti-inflammatory genes. The mechanisms explaining the protective effect of macrophages against renal injury involve inhibition of effector macrophages, effector lymphocytes and proinflammatory cytokines, and the promotion of regulatory T lymphocytes and anti-inflammatory cytokines.
51,52 those exposed to IL-4 and IL-13, called M2a or wound-healing macrophages; those exposed to immune complexes and IL-1β or LPS, termed M2b; and those exposed to IL-10 and TGFβ or glucocorticoids, termed M2c or regulatory macrophages. Although classically activated, pathogenic macrophages (M1) have been characterized well, the differences and inter-relationships among subsets of M2 in terms of specific markers and functions have not been delineated clearly. In mice with unilateral ureteric obstruction, macrophage depletion during progressive inflammatory fibrosis ameliorate scarring and reduce myofibroblast numbers, whereas macrophage depletion during the recovery phase leads to a failure of matrix degradation and persistent scarring, revealing the presence of two distinct macrophage phenotypes, M1 and M2.53 Another study showed that FTY720, an immunosuppressive modulator of sphingosine 1-phosphate receptor, decreases M2 macrophages in 5 of 6 nephrectomized rats.54 We have been unable to demonstrate a unique subset of macrophages that exhibit features of M2 macrophages in normal kidney, in contrast to the demonstration of M2 macrophages in normal intestine55 and lung.56 Whether M2 macrophages exist in diseased kidneys awaits demonstration in both humans and in animal models. Just as the presence of M1 macrophages in damaged kidney does not prove their pathogenic role, so the demonstration of M2 macrophages does not prove that they are mitigating damage.

MACROPHAGES AS A TOOL IN RENAL DISEASES

Although extensive in vitro studies demonstrate that M2 macrophages secrete anti-inflammatory cytokines such as IL-10 and TGFβ and express inhibitory molecules such as indoleamine 2,3-dioxygenase, mannose receptor, and arginine, whether M2 macrophages have an anti-inflammatory role in vivo renal disease is open to question. IFM2 macrophages are to be used as a therapeutic tool in vivo, two conditions must be met: a stable phenotype and the ability to access inflamed sites or local organ draining lymph nodes. Our data demonstrate that after cytokine modulation suppressive features of M2 macrophages are maintained for up to 4 weeks in vitro.57 M2 macrophages express greater numbers of chemokine receptors than resting macrophages and readily accumulate in inflamed kidney and renal draining lymph nodes.

Given their phenotypic stability and ability to access diseased kidney, M2 macrophages have been tested for their ability to modify renal disease in animal models. Our group first examined the role of M2 macrophages in SCID mice with adriamycin nephropathy, a model of human focal segmental glomerulosclerosis, to determine their direct effects on injury in the absence of endogenous T and B cells. In that study, macrophages were isolated from spleen and incubated with IL-4 and IL-13 ex vivo to induce an M2 phenotype (M2a). These macrophages were then infused into SCID mice with adriamycin nephropathy, where they reduced histologic and functional injury.57 The protective effect of M2a macrophages was associated with reduced accumulation and downregulated expression of inflammatory cytokines of endogenous effector macrophages. The regulatory effects of M2 macrophages were target-specific and sustained. Subsequently, we showed a similar protective effect of M2a macrophages against renal structural and functional injury in immunocompetent mice with adriamycin nephropathy. Our group has also investigated the protective effects of M2a macrophages in another model, that of murine streptozotocin-induced (STZ-induced) diabetes. Transfused M2a macrophages accumulate progressively in kidneys for at least 10 weeks after STZ. Kidneys from diabetic mice transfused with M2a macrophages have less glomerular hypertrophy, tubular atrophy, interstitial expansion, and interstitial fibrosis than did diabetic mice not transfused.58 Similarly, M2a macrophage transfusion of diabetic endothelial nitric oxide synthase null (eNOS−/−) mice resulted in less arteriolar hyalinosis and glomerulosclerosis, and a lower systolic BP than in diabetic eNOS−/− mice who were not transfused (Figure 2 and Table 1B).59

THE POTENCY OF DIFFERENT SUBSETS AND DIFFERENT SOURCES OF M2 MACROPHAGES

Another subset of M2 macrophages produced by exposure to cytokines IL-10 and TGFβ (M2c) is known to exhibit anti-inflammatory cytokine production and suppressive functions in vitro; however, its therapeutic efficacy is unproven and relative potency in comparison to M2a macrophages unknown. Studies demonstrating the efficacy of M2c macrophages in reducing injury in murine adriamycin nephropathy have been published recently.60 The relative efficacy of M2a and M2c macrophages were compared in adriamycin nephropathy (unpublished). M2c macrophages display a greater protective effect than M2a macrophages against tubular atrophy, interstitial expansion, and proteinuria. This greater potency of M2c than M2a macrophages could relate to the high-level expression by M2c but not M2a macrophages of the regulatory co-stimulation molecule, B7-H4, and the ability to induce T regulatory cells from CD4+CD25+ T lymphocytes.61

In the above studies, M2 macrophages, produced ex vivo from resting splenic macrophages, ameliorate adriamycin nephropathy. However, the importance of the organ of origin of M2 macrophages is unknown. Therefore, the effects of splenic M2 and bone marrow M2 macrophages induced ex vivo by IL-4/IL-13 (M2a) were compared in adriamycin nephropathy. In vitro, M2a from spleen or bone marrow both showed high-level expression of IL-10 and TGFβ and regulatory molecules, mannose receptor, and arginine. Likewise, they suppress effector macrophages and cytotoxic CD8+ T cells with similar efficacy. However, unlike M2a macrophages derived from spleen, those from bone marrow do not protect against renal structural or functional injury.62 To investigate the failure of
bone marrow M2a macrophages to protect against nephropathy, transfused macrophages (labeled with DiI) were separated from inflamed kidney and examined by cell sorting. Expression of anti-inflammatory cytokines and regulatory molecules by transfused bone marrow M2a macrophages, but not by transfused splenic M2a macrophages, is dramatically reduced in inflamed kidney. The loss of suppressive function of bone marrow M2a macrophages is linked to their proliferation within inflamed kidney. M2a macrophages from bone marrow, but not from spleen, proliferate strongly in kidney, and dividing cells do not express the regulatory phenotype of M2 macrophages.

PROTECTIVE MECHANISMS OF M2 MACROPHAGES

The mechanisms underlying the renoprotective effects of M2 macrophages involve effects on activated macrophages, CD4 T cells, and CD8 T cells. M2 macrophages are also able to suppress classically activated M1 macrophages, which exhibit reduced production of proinflammatory cytokines and iNOS in the presence of M2 macrophages. Macrophage-mediated protection could also involve phagocytosis of damaged cells and cell debris. Our demonstration that M2 macrophages protect against injury in immunodeficient mice with adriamycin nephropathy suggests they act independently of lymphocytes. M2 macrophages produce anti-inflammatory cytokines that inhibit function and proliferation of effector lymphocytes. Both M2a and M2c macrophages are able to inhibit the proliferation of CD4+ T cells in vitro. Moreover, this inhibition is partially blocked by neutralizing antibodies against IL-10, TGFβ, or the co-stimulatory molecule B7-H4 and requires cell-to-cell contact. In vitro and in vivo, M2c, but not M2a, macrophages convert CD4+CD25− T cells to Foxp3+ cells. The mechanism underlying conversion of naïve T cells to T regulatory cells by M2c macrophages depends on high-level expression of B7-H4 by M2c macrophages. Both M2a and M2c macrophages are able to suppress CD8+ T cell–mediated toxicity to tubular cells (Figure 2 and Table 1B).

POTENTIAL DAMAGING EFFECTS OF M2 MACROPHAGES AND THE IMPLICATIONS FOR THERAPY

Macrophages, unlike T cells, which undergo irreversible differentiation upon stimulation, can retain phenotypic plasticity and respond to different environmental signals. Studies indicate that the phenotype of macrophages change over time; for example, macrophages in the earliest stages of cancer resemble classically activated M1 macrophages, yet with tumor growth, those macrophages develop a regulatory phenotype. Similarly, macrophages in adipose tissue of nonobese humans have a wound-healing phenotype, whereas those in obese individuals have a proinflammatory phenotype. It is not clear whether phenotypic deviation occurs in transfused anti-inflammatory macrophages used therapeutically. In our previous study, macrophage phenotype after initial ex vivo cytokine modulation was stable in vitro and little changed in vivo. Recently, we examined macrophages 1 to 3 weeks after transfusion in an immunocompetent model of adriamycin nephropathy; the phenotype of transfused macrophages did drift, but not toward distinct M1 or M2 phenotypes.

M2 macrophages express high levels of TGFβ, a growth factor linked to renal fibrosis. Macrophage-derived TGFβ promotes fibrogenesis by paracrine activation of matrix-producing myofibroblasts and tubular epithelial cell transition into myofibroblasts. However, recent studies in mice with adriamycin nephropathy treated with M2 macrophages show a reduction instead of promotion of renal fibrosis. Interestingly, transfused M2 macrophages gradually decrease their secretion of TGFβ so that transfused M2 macrophages are persistently anti-inflammatory but decreasingly profibrotic with progression of disease. Nevertheless, the possible profibrotic effect of M2 macrophages in late stages of chronic kidney disease is unclear.

In vivo proliferation of transfused M2 macrophages is another potential obstacle to therapeutic application. M2 macrophages separated from bone marrow, but not spleen, proliferate in vitro for up to 3 weeks and in kidneys of mice with adriamycin nephropathy. This proliferation of M2 macrophages associates with loss of their protective phenotype.

FUTURE PERSPECTIVES

Before macrophages can become a therapeutic target or tool for human kidney disease, there needs to be a much greater understanding of the biology of these plastic, phenotypically diverse cells. For example, future studies need to examine the specific phenotype of M2 macrophages in various renal diseases, their interaction with dendritic cells and other intrinsic kidney cells, and their potential to fuse with renal cells to generate new tissue. Macrophage modulation ex vivo to produce protective macrophages is a relatively straightforward and effective approach for treating experimental inflammatory renal disease, and such therapeutic macrophages are target-specific, effective, and sustained in their action. However, relative in vivo efficacy of different subsets of M2 macrophages and their dependence on unique features of various kidney diseases remain unclear. Also, the optimal dose, timing, and frequency of M2 macrophages administration need definition. M2 macrophages are effective when given before or soon after the onset of renal injury, whereas their effect in advanced disease is unproven. One of several hurdles to their consideration as a therapeutic tool for humans is their potency. Potential strategies for enhancing their potency include optimizing culture methods with different cytokine combinations and T regulatory cell contact, potentiation of regulatory gene expression, and antigen exposure to induce antigen specificity.

DISCLOSURES

None.
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