MCP-1/CCL2: a new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy

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THE DEVELOPMENT OF DIABETES involves metabolic, endocrine, and hemodynamic abnormalities which can promote a state of chronic inflammation and vascular dysfunction in many tissues. In the kidney, this can lead to the development of an innate immune response which is predominantly characterized by the accumulation of kidney macrophages (8). Studies in human and experimental diabetic nephropathy have shown that kidney macrophage accumulation is associated with the progression of diabetes (hyperglycemia, HbA1c), the development of renal injury (tissue damage, albuminuria) and kidney fibrosis (myofibroblast accrual, sclerosis), and the decline in renal function, suggesting that it is an inflammatory-mediated disease (8, 9, 33). This concept is supported by animal model studies which have demonstrated that kidney macrophage accumulation is a critical factor in the development of diabetic nephropathy. However, specific anti-inflammatory strategies are not yet being considered for the treatment of patients with diabetic renal injury. This review highlights the chemokine monocyte chemoattractant protein-1 (MCP-1)/CC-chemokine ligand 2 as a major promoter of inflammation, renal injury, and fibrosis in diabetic nephropathy. Researchers have found that diabetes induces kidney MCP-1 production and that urine MCP-1 levels can be used to assess renal inflammation in this disease. In addition, genetic deletion and molecular blocking studies in rodents have identified MCP-1 as an important therapeutic target for treating diabetic nephropathy. Evidence also suggests that a polymorphism in the human MCP-1 gene is associated with progressive kidney failure in type 2 diabetes, which may identify patients at higher risk who need additional therapy. These findings provide a strong rationale for developing specific therapies against MCP-1 and inflammation in diabetic nephropathy.

Kidney Cells Produce MCP-1 in Response to Diabetes

MCP-1 is a secreted protein which specifically attracts blood monocytes and tissue macrophages to its source, via interaction with CCR2, its cell surface receptor (5). Kidney cells produce MCP-1 in response to a variety of proinflammatory stimuli (36), and predictably, its expression has been identified in kidney diseases which involve significant inflammation (37), which include diabetic nephropathy (1, 49). Elements of the diabetic milieu are known to induce MCP-1 mRNA synthesis and protein secretion by cultured renal parenchymal cells, suggesting that the onset of diabetes can provoke renal macrophage recruitment (Fig. 1).

High levels of glucose have been shown to stimulate MCP-1 production by human and mouse mesangial cells through a pathway which involves activation of PKC, increased levels of oxidative stress, and the activation/nuclear translocation of the transcription factor nuclear factor-κB (NF-κB) (20, 24, 55). This stimulatory effect of high glucose in mesangial cells is further enhanced by the presence of advanced glycation end products (AGEs) or mechanical stretch (2, 17). A variety of AGEs are also capable of stimulating MCP-1 production by mesangial cells, which involves interaction with the receptor for AGEs (RAGE) with subsequent generation of oxidative stress via activation of peroxisome proliferator-activated receptor-γ (PPARγ) (2, 29, 53).

Kidney epithelial cells, including glomerular podocytes and tubular cells, also make MCP-1 in response to high glucose and...
Diabetic Milieu
High Glucose
AGEs

Monocytes
Activated Macrophages

MCP-1
ROS, proinflammatory cytokines
proliferative growth factors

Parenchymal Cells
Injury
Apoptosis, Necrosis
Inflammation
Fibrosis

Myofibroblasts
Matrix Deposition
Progression of Diabetic Nephropathy

Blood
Kidney

MCP-1

Fig. 1. Postulated role for monocyte chemoattractant protein-1 (MCP-1) in diabetic nephropathy. Elements of the diabetic milieu induce renal parenchymal cells to secrete MCP-1, which attracts monocytes into the kidney and stimulates myofibroblast-like properties in mesangial cells. Further exposure of kidney macrophages to MCP-1 and the diabetic milieu promotes macrophage activation, resulting in the release of reactive oxygen species (ROS), proinflammatory cytokines (e.g., IL-1, TNF-α), MCP-1, and proliferative growth factors (e.g., PDGF, TGF-β). The self-amplifying inflammatory response causes injury and death to parenchymal cells, and the fibrotic response induces myofibroblast proliferation and enhanced production of extracellular matrix by fibroblasts and mesangial cells. Together, these responses promote the progression of diabetic nephropathy, resulting in the development of renal failure.

AGEs. Exposure to high glucose rapidly induces MCP-1 mRNA and protein release by cultured mouse podocytes, which is inhibited by treatment with all-trans retinoic acid (22). Glycated BSA and carboxymethyllysine (CML)-BSA also stimulate MCP-1 gene and protein expression in mouse podocytes, which occurs through a mechanism involving RAGE, oxidative stress, and activation of extracellular signal-regulated kinase (ERK) and NF-κB and is inhibited by pravastatin (18, 19). Similarly, cultured rat proximal tubular cells secrete increased levels of MCP-1 in response to both high glucose and AGE-BSA (11).

Urine MCP-1 Levels Correlate With Kidney Macrophage Accumulation in Diabetic Nephropathy

Analysis of patient biopsies and animal models by in situ hybridization or immunostaining has identified expression of MCP-1 mRNA and protein in diabetic kidneys (glomerular podocytes, mesangial cells, and tubules), which correlates with the accumulation of CD68+ macrophages (8, 11, 49). Animal model experiments also show that kidney MCP-1 is increased early in disease, being most abundant in tubules, and progresses with the development of diabetic nephropathy (11). Serum levels of MCP-1 are sometimes elevated in diabetic patients; however, this is not associated with the development of albuminuria, kidney macrophage accumulation, or nephropathy (2, 28, 49). In contrast, urine levels of MCP-1 closely reflect kidney MCP-1 production and correlate significantly with levels of albuminuria, serum glycated albumin, urine N-acetylglucosaminidase (NAG), and kidney CD68+ macrophages in human and experimental diabetic nephropathy (2, 11, 12, 32, 49). This has led to the suggestion that proteinuria during diabetes may itself aggravate tubular injury and accelerate nephropathy by increasing tubular MCP-1 production and the inflammatory response. This concept is supported by in vitro and in vivo studies indicating that protein overload can induce tubular expression of MCP-1 (13, 44). However, during diabetes, it appears more likely that tubular MCP-1 is initially induced by the diabetic milieu, since increases in tubular MCP-1 and interstitial macrophages coincide with the development of hyperglycemia and precede a rise in albuminuria in type 1 diabetic nephropathy in mice (11). In comparison, a rat albumin overload model, which develops instant proteinuria, takes 2 wk to induce an increase in kidney MCP-1 mRNA levels (13), suggesting that excreted forms of albumin cannot independently promote rapid tubular production of MCP-1 in vivo.

Urinary levels of MCP-1 can be used to assess the efficacy of treatments in reducing diabetic renal inflammation. Treatment of patients with the angiotensin-converting enzyme (ACE) inhibitor lisinopril reduced urine MCP-1 in type 2 diabetic nephropathy, and this correlates with the decline in proteinuria (1). Similarly, type 2 diabetic patients with nephropathy have elevated urine MCP-1 levels, which correlate with urine 8-iso-prostaglandin F$_{2α}$ (a marker of renal oxidative stress), and both of these indicators are decreased by aldosterone blockade with spironolactone (45). In a rat model of type 1 diabetic nephropathy, treatment with retinoic acid reduces urine MCP-1, which correlates with a decline in immunostaining for MCP-1 and CD68+ macrophages in the kidney (22). These findings suggest that urine MCP-1 may have significant diagnostic value in evaluating the renal inflammatory response in patients with diabetic nephropathy. However, whether this will be better than albuminuria at predicting diabetic renal inflammation is unknown. It is possible that urinary levels of MCP-1 may respond more quickly to anti-inflammatory therapies and predict later changes in albuminuria, which could be clinically useful.

**MCP-1 Promotes Inflammation and Progressive Injury in Diabetic Kidneys**

In overt diabetic nephropathy, the predominant source of MCP-1 is the cortical tubules (11), which is similar in other inflammatory kidney diseases (46, 47). Macrophage accumulation around these tubules is associated with tubular injury and myofibroblast accumulation, which lead to declining renal function (8, 9) (Fig. 1). The importance of MCP-1 in the early development of diabetic nephropathy has been determined using animal models incorporating genetically deficient mice or therapeutic blockade of the MCP-1 receptor (CCR2) (11, 12, 25). In a model of streptozotocin-induced type 1 diabetic nephropathy, mice genetically deficient in MCP-1 were found to have reduced renal injury compared with wild-type mice with equivalent hyperglycemia (11). In this study, the absence of MCP-1 resulted in the prevention of albuminuria and elevated plasma creatinine at 18 wk of diabetes, which coincided with a marked reduction (≥50%) in glomerular and interstitial CD68+ macrophages and histological damage (11). In addition, a smaller percentage of macrophages in MCP-1-deficient diabetic kidneys had co-expression of CD169 and inducible nitric oxide synthase (iNOS), suggesting that these macrophages were less activated (11). Therefore, this study demonstrates that MCP-1 promotes
macrophage recruitment and activation and the development of renal injury in diabetic kidneys. Similar findings have also been obtained in the *db/db* mouse model of type 2 diabetic nephropathy. MCP-1-deficient *db/db* mice are not protected from the development of obesity, adipose inflammation or type 2 diabetes (12). However, at 32 wk of age, these *db/db* mice have striking reductions (≥50%) in albuminuria, plasma creatinine, macrophage recruitment, and activation and histological injury compared with their wild-type diabetic controls (12). These findings obtained from diabetic nephropathy studies in MCP-1-deficient mice have recently been supported by a study using both pharmacological treatment and gene therapy to block MCP-1 function in mice which spontaneously develop type 1 diabetes (25). In this study (25), MCP-1 action was blocked in diabetic mice for 12 wk by either food supplementation with propagermanium (a CCR2 antagonist) or muscle transfection with a plasmid containing the 7ND gene (a mutant of MCP-1). Both of these strategies were found to reduce glomerular macrophage infiltration and glomerulosclerosis during diabetes; however, the effect on albuminuria was not reported.

In vitro evidence also indicates that MCP-1 may promote macrophage activation in diabetic kidneys (Fig. 1). Stimulation with MCP-1 rapidly induces the activation of ERK and JNK signaling pathways in cultured macrophages, which are known promoters of the macrophage inflammatory response (42). Furthermore, blockade of ERK activity with PD98059 prevents MCP-1-stimulated macrophage activity by inhibiting actin polymerization and TNF-α production (42). In addition, MCP-1 induces iNOS mRNA and nitric oxide release by peritoneal macrophages, which is dependent on signaling through phosphoinositide-3-kinase, PKC, and ERK (4). These data support a direct role for MCP-1 in macrophage activation; however, it is unclear whether this will play an important role in the diabetic kidney compared with the activation induced by high glucose, AGES, oxidative stress, and proinflammatory cytokines.

**MCP-1 Stimulates Renal Fibrosis in Diabetic Kidneys**

Evidence from animal models and in vitro experiments suggests that MCP-1 can directly and indirectly promote renal fibrosis during diabetes (Fig. 1). The initial studies of MCP-1-deficient diabetic kidneys showed that a lack of MCP-1 reduces the accumulation of interstitial myofibroblasts and the deposition of glomerular and interstitial collagen type IV (11, 12). Subsequent examination has shown that MCP-1 deficiency in type 1 diabetes also reduces glomerular deposition of fibronectin as well as mRNA and protein expression of fibronectin and transforming growth factor-β1 (TGF-β1) in the total kidney (16). In addition, therapeutic blockade of MCP-1 in type 1 diabetic mice reduces glomerular deposition of TGF-β1 and collagen IV and the mesangial matrix fraction (25). Notably, in each of these studies, a deficiency or blockade of MCP-1 was associated with a marked decline in the number of kidney macrophages, suggesting that MCP-1-mediated fibrosis may be due to the recruitment of macrophages.

Macrophages recruited into a diabetic environment can promote renal fibrosis. Culturing macrophages in the presence of diabetic serum or AGES causes them to release interleukin-1 and platelet-derived growth factor, which induces the proliferation of renal interstitial fibroblasts (9). In addition, MCP-1 can directly stimulate macrophages to secrete increased levels of active and total TGF-β1, which can subsequently promote production of extracellular matrix (50). Therefore, MCP-1 has the ability to influence the macrophage contribution to fibrosis through both direct and indirect mechanisms.

MCP-1 is also capable of inducing a fibrotic response in glomerular mesangial cells. MCP-1 signaling through CCR2 on human mesangial cells has been shown to induce fibronectin mRNA and protein synthesis by a mechanism involving TGF-β1 production and activation of NF-κB in these cells (16). This may have importance in diabetic glomerulosclerosis, since MCP-1 can promote glomerular TGF-β1 production without affecting glomerular macrophage accumulation (39). Furthermore, TGF-β1 can itself stimulate mesangial expression of MCP-1, which suggests the possibility of a self-amplifying cytokine-loop mechanism for promoting mesangial matrix accumulation (7).

**MCP-1/CCR2 Gene Polymorphisms and Diabetic Nephropathy**

Studies examining the influence of MCP-1 gene polymorphisms on the progression of kidney disease have focused on the -2518 A/G single-nucleotide polymorphism (SNP) in the distal regulatory region of MCP-1 which is believed to regulate gene expression. This SNP was originally identified as potentially important when it was shown that presence of a specific allele was associated with greater production of MCP-1 by interleukin-1β-stimulated peripheral blood mononuclear cells (38). Subsequent clinical studies have since shown that the G allele, which is more highly expressed in Asian and Mexican individuals compared with Caucasians (38), is associated with a reduced susceptibility to renal injury in some studies of lupus nephritis and IgA nephropathy (26, 31). However, the association of these particular kidney diseases with MCP-1 polymorphisms remains controversial.

The presence of the MCP-1 A(-2518) allele has also been associated with the development of diabetes and diabetic nephropathy. Frequencies of the A allele and A/A genotype are reported to be significantly higher in Caucasian patients with type 1 diabetes compared with normal controls (54). Similarly, in a large cohort of Caucasians, the G allele correlates with a lower incidence of type 2 diabetes and is associated with decreased levels of plasma MCP-1 (41). In contrast, a study performed in Korean patients showed no significant difference in the overall distribution of -2518 A/G in the MCP-1 gene in patients with type 2 diabetes compared with healthy controls (30). However, this study demonstrated a significant association of the A allele with diabetic kidney failure, suggesting that in some populations, the A allele may be more related to the development of diabetic nephropathy than the occurrence of diabetes itself.

Current evidence suggests that there are no associations of SNPs in CCR2 with the development of diabetic nephropathy or other kidney diseases. One study in children has identified an increased frequency of G-to-A substitution in the CCR2 gene at position 190 (CCR2–64I) which is associated with the development of type 1 diabetes (43), but subsequent studies have been unable to confirm this finding in adults or show any associations with diabetic complications (54).
**Invited Review**

**MCP-1/CCL2 IN DIABETIC NEPHROPATHY**

**Therapeutic Strategies Targeting MCP-1 in Diabetic Nephropathy**

Despite current treatments including glycemic control, ACE inhibition, angiotensin receptor blockers, and statins, diabetic nephropathy continues to progress in many patients in association with kidney macrophage accumulation, suggesting the need for additional immunotherapy (33). In support of this concept, a recent study in rats has shown that immunosuppression with mycophenolate mofetil provides added benefits when combined with ACE inhibition in treating diabetic nephropathy (52). In this study, combined treatment produced superior suppression of kidney macrophage recruitment in association with greater reductions in renal MCP-1 and TGF-β1. This suggests that specific targeting of MCP-1 would be beneficial as an adjunct therapy to patients with diabetic nephropathy.

Several novel therapies have recently been shown to indirectly reduce kidney expression of MCP-1 and diabetic renal inflammation in animal models by targeting renal oxidative stress or the downstream intracellular signaling pathways, which are induced by hyperglycemia. These include the use of an analog of 1,25-dihydroxyvitamin D₃ (55), a PPARγ agonist (3), a PKC-β inhibitor (51), eicosapentaenoic acid (21), and a flavonoid (34). However, the suppression of the inflammatory response was only partially affected by these treatments, indicating the need for more specific blockade of MCP-1.

The essentially normal phenotype of MCP-1-deficient mice suggests that the use of anti-MCP-1 therapies in chronic diseases will not be harmful (11). There are a number of strategies that selectively target MCP-1 which have been proven effective in rodent models of inflammatory disease. Small molecular antagonists of CCR2 (INCB3344, proggermanium, RS-504393) have been shown to suppress inflammation in mouse models of multiple sclerosis, renal ischemia-reperfusion injury, urter obstruction, and diabetic nephropathy and in a rat model of arthritis (6, 15, 25, 27). Engineered biological antagonists of CCR2 have also proven effective (14, 23, 40). Subcutaneous infusion of cells transduced with a vector expressing a truncated inactive form of MCP-1 has been found to suppress the development of renal inflammation in a mouse model of lupus nephritis (23). Similarly, muscle transfection with 7ND (a mutant of MCP-1) reduces renal inflammation in mouse models of renal ischemia-reperfusion injury, lupus nephritis, and diabetic nephropathy (14, 40, 25). These studies provide the foundation for the development of specific anti-MCP-1 therapies to treat human diseases, including diabetic nephropathy.

**Conclusions**

Evidence from human studies and animal models demonstrates that kidney MCP-1 production plays a critical role in the development of diabetic renal inflammation, which leads to progression of diabetic nephropathy. Current therapies can only partially reduce the impact of MCP-1 on this disease, suggesting the need for additional treatment. Urinary levels of MCP-1 can be monitored as a marker of diabetic renal inflammation, and this may prove to have significant diagnostic value in assessing the effectiveness of novel or combined therapies. Selective targeting of MCP-1 has been shown to be an effective treatment in suppressing animal models of kidney disease which include diabetic nephropathy; however, such therapies have not yet been validated in human diabetic nephropathy. Given that macrophages also play a significant role in the development of nonrenal diabetic complications (48), it is feasible that any new therapies targeting MCP-1 may have broader clinical importance than treating diabetic nephropathy alone.

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