

## In-Depth Topic Review

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# Chemokine Receptor CCR1: A New Target for Progressive Kidney Disease

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**Key Words**

Chemokines · Inflammation · Kidney disease · Fibrosis

**Abstract**

Infiltrating leukocytes are thought to contribute to the progression of kidney disease. Locally produced chemokines guide circulating leukocytes into the kidney, which renders therapeutic blockade of respective chemokine receptors on the leukocyte surface as potential targets for the inhibition of renal leukocyte recruitment. By using mutant mice and specific antagonists, we found that chemokine receptor CCR1 has non-redundant functions for leukocyte adhesion to activated vascular endothelium and for transendothelial diapedesis. Most importantly, CCR1 blockade with a specific small molecule antagonist can improve injury in several types of progressive kidney disease models, even if treatment is initiated in advanced disease states. Identification of new targets may add to the therapeutic options in chronic kidney disease.

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**Introduction**

The global burden of chronic kidney diseases remains an ongoing medical challenge. Therapies that can halt or reverse advanced renal injury are not available. Increasing numbers of patients progress to end-stage renal failure

and require renal replacement therapy, the latter being associated with significant mortality, a lower quality of life, and high costs for national health systems. Thus, new treatment strategies that slow down, halt or even revert progressive renal damage are requested. Current treatments for chronic renal failure target to correct renal hemodynamics, proteinuria, high blood pressure, hyperparathyroidism or to avoid nephrotoxic drugs and nicotine abuse. A general role of immunosuppressive drugs in chronic kidney disease is not established, because of lack of efficacy or unacceptable side effects of systemic immunosuppression.

Many types of chronic kidney diseases are characterized by an accumulation of interstitial leukocytes. Infiltrating leukocytes are a major source for proinflammatory and profibrotic cytokines and are therefore critical for mediating fibroblast proliferation, differentiation into myofibroblasts, matrix production, and tubular atrophy [1]. Thus, interfering with renal leukocyte recruitment may represent a valuable strategy to reduce renal inflammation, tissue remodeling, and progressive loss of renal function. During the past decade we and others have unraveled the role of chemokines and chemokine receptors that direct circulating leukocytes into the damaged kidney, which was recently reviewed in detail [2]. In this article, we focus on chemokine receptor CCR1 that was identified from a group of other chemokines to play a critical role for interstitial leukocyte recruitment.

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**Table 1.** Classification of chemokines and chemokine receptors

Ligand		Receptors	Ligand		Receptors
CXCL1	Gro $\alpha$	CXCR1, 2	CCL1	I-309	CCR8
CXCL2	Gro $\beta$		CCL2	MCP-1	CCR2
CXCL3	Groy	CXCR1, 2	CCL3	MIP-1 $\alpha$	CCR1, 5
CXCL4	PF4		CCL4	MIP-1 $\beta$	CCR5
CXCL5	ENA-78	CXCR2	CCL5	RANTES	CCR1, 3, 5
CXCL6	GCP-2	CXCR1, 2	CCL7	MCP-3	CCR1, 2
CXCL7	NAP-2	CXCR2	CCL8	MCP-2	CCR1, 2, 5
CXCL8	IL-8	CXCR1, 2	CCL11	Eotaxin	CCR3
CXCL9	Mig	CXCR3	CCL13	MCP-4	CCR1, 2, 3
CXCL10	IP-10	CXCR3	CCL14	HCC-1	CCR1
CXCL11	I-TAC	CXCR3	CCL15	HCC-2	CCR1
CXCL12	SDF-1	CXCR4	CCL16	HCC-4	CCR1, 8
CXCL13	BCA-1	CXCR5	CCL17	TARC	CCR4
CXCL14	Bolekine		CCL18	DC-CK1	
CXCL15	Lungkine		CCL19	ELC	CCR7
			CCL20	LARC	CCR6
<i>XCL</i>			CCL21	SLC/6Ckine	CCR7
XCL1	Lymphotactin	XCR1	CCL22	MDC	CCR4
XCL2	SCM-1 $\beta$		CCL23	MPIF-1	CCR1
			CCL24	Eotaxin-2	CCR3
			CCL25	TECK	CCR9
<i>CX3CL</i>			CCL26	Eotaxin-3	CCR3
CX3CL1	Fractalkine	CX3CR1	CCL27	CTAK/Eskine	CCR10

### Chemokines and Chemokine Receptors in Renal Leukocyte Recruitment

#### *Chemokines and Chemokine Receptors*

Chemokines are a large family of low molecular weight cytokines that cause migration of leukocytes. The nomenclature used to describe individual chemokine ligands is based upon the four-cysteine motif in their amino acid sequence as listed in table 1 [3]. Chemokines can be further classified according to their predominant function and expression pattern. For example, chemokines such as CCL21 or CCL19 are classified as homeostatic chemokines. They are involved in physiological homing of leukocytes to lymphoid tissues [4, 5], and in lymphocyte and dendritic cell trafficking during immune surveillance [6]. Chemokines such as CCL2, CCL3, CCL5, and CXCL10 are classified as proinflammatory chemokines because they mediate the recruitment of leukocytes to sites of tissue injury [7, 8]. Chemokines can interact with only one receptor or a single chemokine binds to multiple receptors [8].

Chemokines mediate their biologic actions through a family of more than 20 seven-transmembrane-spanning G-protein-coupled receptors [4, 9, 10]. Chemokine recep-

tors are designated according to the class of their chemokine ligands, e.g. CR, CCR, CXCR, and CX3CR (table 1). Chemokine receptors show restricted expression on subclasses of leukocytes. Because the ligand specificities of the receptors can substantially overlap within a chemokine class, a high degree of redundancy of single chemokines was suspected [5, 8]. In general, the proinflammatory chemokine receptors have promiscuous ligand binding specificities. This finding gave rise to the concept that blocking chemokine receptors could represent a rational approach to target the chemokine system rather than targeting a single chemokine.

#### *Chemokines and Chemokine Receptors Mediate Leukocyte-Endothelial Cell Interactions*

Chemokines are involved in the interactions of leukocytes and activated endothelial cells at multiple stages [5, 11]. Injured renal cells produce inflammatory mediators that enhance the expression of adhesion molecules on endothelial cells of capillaries adjacent to the inflammatory lesion. Selectins mediate the rolling process of leukocytes along the endothelial surface and thereby allow contact of leukocytes with chemokines. The Duffy antigen receptor for chemokines (DARC) can bind chemokines

at post-capillary venules [12]. However, in various forms of renal diseases, DARC expression expands to peritubular vessel endothelium and thereby marks specific exit sites for leukocytes into the renal interstitium [12]. Chemokines ligate their respective receptors on the surface of the rolling leukocyte. This activates leukocyte-expressed  $\beta_2$ -integrins, resulting in firm adhesion of the leukocyte to the endothelial surface as a prerequisite for leukocyte transmigration [5, 7, 13]. Other chemokine receptors appear to differentially influence spreading, diapedesis, and subsequent migration into the tissue space [5, 14]. For example, monocytes and T cells express both chemokine receptors CCR1 and CCR5 [15]. In an in vitro system it was found that immobilized CCL5 induced leukocyte arrest via CCR1, while leukocyte spreading was mediated via CCR5, and transendothelial migration was supported by both receptors [15]. In this context it is of interest that chemokines also modulate the redistribution of junctional adhesion molecules at endothelial cell tight junctions that may promote leukocyte diapedesis by transient opening of focal cell-cell contacts [16]. Furthermore, CCL5 up-regulates the secretion and activity of matrix metalloproteinases by infiltrating leukocytes [17], thus facilitating leukocyte transmigration through the basement membrane and extracellular matrix [18].

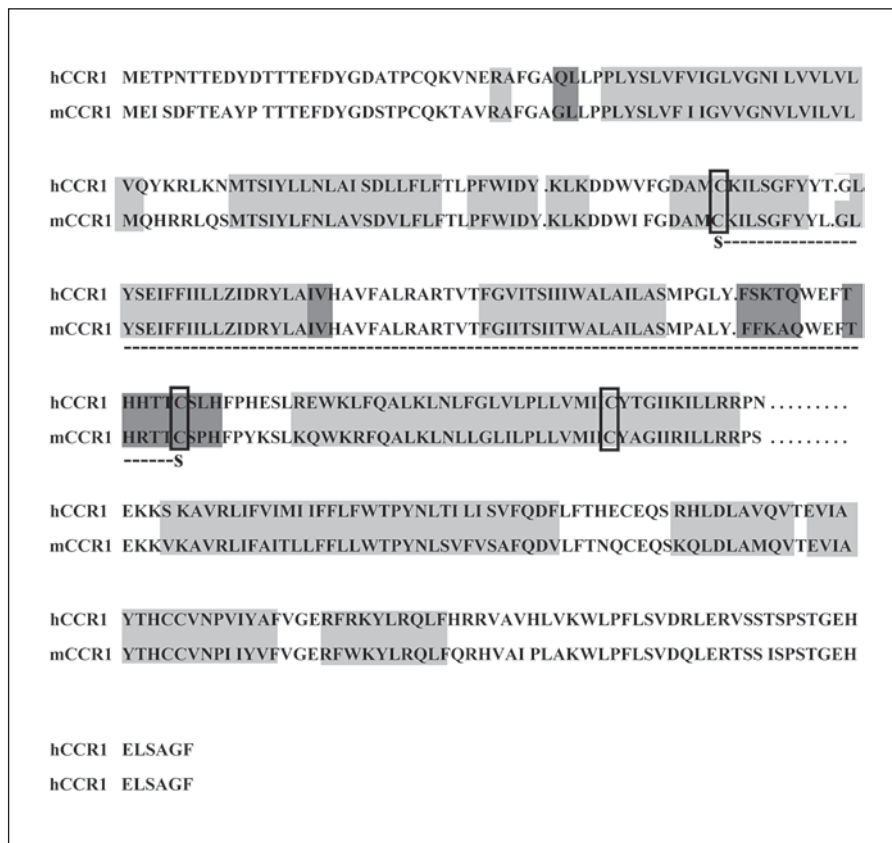
#### *Chemokines and Chemokine Receptors in Renal Inflammation*

All types of renal cells can produce chemokines upon stimulation [reviewed in 11]. Proinflammatory stimuli including reactive oxygen species, growth factors and vasoactive agents like angiotensin II can stimulate chemokine production of renal cells. Furthermore, immune complexes and complement activation cause mesangial production of chemokines. In proximal tubular cells, chemokines can be induced by LPS [19], high concentrations of albumin [20, 21] or exposure to both calcium oxalate and calcium phosphate crystals [22]. Besides intrinsic renal cells, infiltrating leukocytes are a major source of local chemokine production in a positive amplification loop [23, 24], as chemokines secreted by infiltrating leukocytes promote additional leukocyte recruitment [25]. It is important to note that chemokine expression is restricted to the injured compartment of the kidney [reviewed in 26]. The spatial expression of chemokines in the kidney correlates with the local accumulation of inflammatory cell infiltrates and renal damage [2, 24]. Data from animal models have been confirmed by human renal biopsy studies [12, 27–29].

Termination of the trigger injury correlates with a reduction of chemokine expression by intrinsic renal cells and infiltrating leukocytes [30]. As further influx of leukocytes does not occur, the number of infiltrating leukocytes declines in parallel to the resolution of disease. It is important to note that termination of the chemokine signal is critical for the resolution of the inflammatory process. If local chemokine expression is augmented by another trigger of chemokine release, a pre-existing renal disease may eventually progress to severe renal damage. For example, intercurrent infections frequently result in a deterioration of renal diseases including chronic transplant nephropathy. The proinflammatory signals of bacterial and viral invasion are mediated by Toll-like receptors (TLRs) [31]. TLRs recognize pathogen-associated molecules such as LPS, peptidoglycans, and unmethylated CpG-DNA [31]. We recently found that injection of unmethylated CpG-DNA into mice with otherwise self-limiting immune complex glomerulonephritis resulted in progression instead of resolution of the disease process. This was associated with increased chemokine expression and subsequent glomerular macrophage recruitment [32]. Even if the triggering injury subsides, renal chemokine expression can be maintained by other mechanisms such as infection, renin-angiotensin activation, hypoxia or proteinuria, and contribute to persistent leukocyte infiltration and tissue damage. Many studies addressed the functional role of single chemokines or chemokine receptors in defined renal disease models by applying either neutralizing antibodies, DNA vaccination, chemokine receptor antagonists, or by using mutant mice [reviewed in 24]. Among those, only a few studies have administered specific antagonists late in the disease process, which most appropriately mimics treatment of established kidney disease. Such data is only available for specific blockade of CCR1.

#### *Identifying CCR1 as a Target*

Human CCR1 binds several CC chemokines, including CCL3 (table 1). The amino acid sequence of human CCR1 has a high degree of homology to murine CCR1 (fig. 1). However, species-specific pharmacodynamics need to be defined for each antagonist when to be tested in another species [33]. The latter often compromises the interpretation of data generated in rodents that apply chemokine antagonists designed for the human system. CCR1 is expressed at low levels on T cells. By contrast, human and murine blood monocytes, tissue macrophages, neutrophils, and eosinophils express CCR1 at high levels [3, 29, 34]. Upon ligation with its ligands, a conforma-



**Fig. 1.** Alignment of human and mouse CCR1. Known or predicted helices are highlighted in light gray. Known or predicted  $\beta$ -strands are highlighted in dark gray. Cysteine residues are encircled. Disulfide bonds is indicated by the letter 'S'.

tional change of the seven-transmembrane elements of CCR1 leads to intracellular activation of G-protein subunits. Studies using an in vitro flow chamber system first identified a critical role for CCR1 for adhesion of rolling macrophages or T cells to activated endothelium using established human cell lines [15]. These findings were validated in vivo by two approaches. First, we used intravital microscopy of the cremaster muscle in mice to study the role of CCR1 for leukocyte rolling, firm adhesion, transendothelial migration, and interstitial migration. Either by applying a specific CCR1 antagonist or performing intravital microscopy in CCR1-deficient mice, we found that CCR1 is required for leukocyte adhesion and transendothelial migration during the recruitment process [35]. The coherent findings in antagonist-treated mice or CCR1-deficient mice argue for a non-redundant role of CCR1 in that processes. Because organ-specific roles of chemokines and chemokine receptors have been reported, studies performed on cremaster muscles do not allow a conclusion upon the role of CCR1 in the kidney. Thus, as a second approach we isolated macrophages and T cells from spleens of CCR1-deficient or wild-type mice.

After ex vivo labeling with a fluorescence dye, cells were injected into mice with renal fibrosis after unilateral ureteral obstruction (UUO) [36]. CCR1-deficient macrophages and T cells showed markedly reduced recruitment to the interstitial compartment of diseased kidneys as compared to cells isolated from wild-type mice [36]. These data show that CCR1 on macrophages and T cells is required for interstitial leukocyte recruitment in renal fibrosis in mice.

#### Compartment-Specific Role of CCR1

Human renal biopsy studies have localized renal CCR1 expression to macrophages in glomerular and interstitial inflammatory cell infiltrates in several types of kidney disease [29]. However, the presence of a receptor found on cells that infiltrate the kidney does not necessarily provide evidence for its functional role in the recruitment process. In fact, organ- and even compartment-specific roles of chemokine receptors may occur. When we treated autoimmune MRL<sup>lpr/lpr</sup> mice with lupus nephritis with BX471, a small molecule CCR1 antagonist, we noted that despite a marked reduction of interstitial leukocyte

counts, the number of glomerular macrophages was not affected compared to vehicle-treated MRL<sup>lpr/lpr</sup> mice [37]. Cell transfer studies with labeled leukocytes revealed that CCR1, while critical for interstitial leukocyte recruitment, is not required for their recruitment into the glomerular compartment of nephritic MRL<sup>lpr/lpr</sup> mice. We obtained similar results in BALB/c and BKS mice with glomerular pathology. By contrast, we could show that CCR5 mediates glomerular but not interstitial macrophage recruitment [23, 36]. Given the fact that CCR1 and CCR5 are present on circulating monocytes and T cells, other factors appear to determine their selective roles for recruitment in different microvascular beds. The interaction of chemokines with their receptors requires additional molecules, e.g. respective binding sites on endothelial cell proteoglycans or DARC that expose the chemokine to the luminal endothelial cell membrane. If endothelial cells of glomerular or peritubular capillaries expose different chemokines in mice, and if these observations hold true for the human kidney, remains a future goal of our ongoing research activities in this field.

### **Late Onset CCR1 Antagonism with a Small Molecule CCR1 Blocker in Models of Progressive Kidney Disease**

#### *Acute and Chronic Renal Allograft Rejection*

The first study that used the small molecule CCR1 antagonist BX471 in kidney disease was reported by Horuk et al. [38] in 2001. In this study, BX471 monotherapy had beneficial effects on serum creatinine levels and renal survival in a model of kidney transplantation in rabbits. Pathologic analysis showed that BX471 was similar to cyclosporine in its ability to prevent extensive infarction of transplanted kidneys [38]. Furthermore, BX471 prevented chronic allograft nephropathy in a Fischer 344 into Lewis rat model of acute and chronic allograft rejection [39]. BX471 given from day 21 to 42 after kidney transplantation reduced the number of ED-1-positive macrophages in renal allografts in association with a reduction of markers of renal fibrosis.

#### *Obstructive Nephropathy*

Experimental UUO represents a model for obstructive nephropathy but also allows insight into the process of interstitial fibrosis that is a common characteristic of many chronic nephropathies. UUO kidneys show increased CCR1 expression as compared to their respective non-obstructed contralateral kidneys [40]. UUO

kidneys from mice treated with the CCR1 antagonist BX471 revealed a marked reduction of interstitial leukocyte counts [41]. Markers of renal fibrosis, such as interstitial fibroblasts, interstitial volume, mRNA and protein expression for collagen I, were all significantly reduced by BX471 compared to vehicle-treated controls. Most interestingly, the beneficial effect was comparable when BX471 was given not before day 6, indicating that late onset of CCR1 blockade may still be effective. By contrast, treatment was ineffective when the drug was supplied only from day 0 to day 5. These data were confirmed by inducing UUO in CCR1-deficient mice [36]. Thus, we decided to further evaluate CCR1 as a potential target for reducing interstitial leukocyte recruitment and fibrosis as major factors for the process of progression to end-stage renal failure.

#### *Immune Complex Glomerulonephritis*

Chemokines are also involved in systemic immune responses [3], so that data from the UUO model may not apply to renal manifestations of systemic autoimmunity, e.g. lupus nephritis. In fact, lack of CCR1 has been reported to be associated with an enhanced Th1-like immune response and aggravation of nephrotoxic serum nephritis [42]. We therefore studied the effects of therapeutic CCR1 blockade in progressive lupus-like immune complex glomerulonephritis of MRL<sup>lpr/lpr</sup> mice. BX471 treatment initiated late during the course of disease (weeks 20–24 of age) improved blood urea nitrogen levels and reduced the amount of macrophages and lymphocytes in the interstitium [37]. Furthermore, BX471 reduced the extent of interstitial fibrosis as evaluated by interstitial smooth muscle actin expression and collagen I deposits, as well as mRNA expression for collagen I and TGF- $\beta_1$ . BX471 did not affect serum DNA autoantibodies despite potential roles of CC chemokines and their receptors in systemic immune responses. As CCR1 blockade does not reduce glomerular macrophage recruitment, it was found to be ineffective in modulating glomerular pathology and proteinuria in MRL<sup>lpr/lpr</sup> mice.

#### *Focal Segmental Glomerulosclerosis*

Proteinuria represents a major prognostic factor for the progression of renal disease, because unselective proteinuria can induce chemokine expression in renal tubular cells [43]. Thereby proteinuria serves as a major factor for tubulointerstitial inflammation. Thus, we questioned whether CCR1 antagonism would be able to improve interstitial fibrosis in the presence of massive proteinuria caused by focal segmental glomerulosclerosis (FSGS).

FSGS was induced in BALB/c mice by two intravenous injections of adriamycin at day 0 and 14. BX471 was started from day 14 when nephrotic syndrome was established. Again, BX471 reduced the amount of interstitial macrophages and T cells and markers of renal fibrosis including interstitial fibroblasts and interstitial volume [44]. These findings demonstrate that therapeutic CCR1 blockade is effective in the presence of heavy proteinuria. Consistent with our previous findings, BX471 did not affect glomerular pathology in adriamycin-injected BALB/c mice.

#### *Glomerulosclerosis in Alport Nephropathy*

Alport syndrome is a common type of hereditary glomerulopathy leading to glomerulosclerosis with subsequent interstitial fibrosis progressing to end-stage renal disease. Disease progression has been thought to be mediated mostly by non-inflammatory factors, but recent studies have documented the role of interstitial macrophages for tubular atrophy in collagen4A3<sup>-/-</sup> mice, with renal lesions comparable to humans [45]. We treated collagen4A3<sup>-/-</sup> mice with BX471 or vehicle beginning from 6 weeks of age for 4 weeks [35]. Vehicle-treated collagen4A3<sup>-/-</sup> mice showed a mean survival of 69 days (95% confidence interval, 64–74 days), whereas daily treatment with BX471 from week 6 increased mean survival to 86 days (95% confidence interval, 80–92 days;  $p = 0.0002$ ). BX471 treatment was associated with less interstitial macrophages, apoptotic tubular epithelial cells, tubular atrophy, interstitial fibrosis, and less globally sclerotic glomeruli as compared to vehicle-treated mice. In contrast, BX471-treated collagen4A3<sup>-/-</sup> mice showed a higher density of peritubular capillaries. Obviously, blocking CCR1-mediated interstitial macrophage recruitment can maintain peritubular microvasculature and prevent tubular atrophy, two major factors for disease progression in inflammatory and non-inflammatory types of progressive nephropathies. These data also indicate the crucial role of macrophage infiltrates for the progression of chronic kidney disease.

#### *Diabetic Nephropathy*

Diabetic nephropathy is the most common type of chronic kidney disease progressing to renal failure. Available rodent models of diabetic nephropathy are frequently used to study the early glomerular changes of diabetic nephropathy [46]. However, rodents usually do not develop advanced interstitial lesions as they occur in late stages of human diabetic nephropathy [47]. Recently, Chow et al. [48] reported that db/db mice develop sig-

nificant interstitial macrophage infiltrates at 6 months of age. We injected male db/db mice from 5 months of age for 10 days and obtained renal tissue for histopathological analysis. BX471 reduced the number of macrophages in the renal interstitium as compared to vehicle-treated db/db mice [unpubl. observation], supporting the hypothesis that blocking interstitial macrophage recruitment with CCR1 antagonists may be beneficial also in diabetic nephropathy.

In summary, CCR1 blockade can effectively prevent recruitment of monocytes and lymphocytes into the renal interstitium. BX471 is effective in multiple models of progressive kidney disease in mice even when treatment was started late in the disease process. Thus, interfering with renal leukocyte recruitment by targeting CCR1 may represent a promising strategy to prevent disease progression in chronic nephropathies characterized by interstitial leukocytic cell infiltrates.

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