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# Chemokines and chemokine receptors as therapeutic targets in chronic kidney disease

**Volker Vielhauer[1], Hans-Joachim Anders[1]**

*[1]Medizinische Poliklinik, Klinikum der Universitat Munchen, Campus Innenstadt, Ludwig-Maximilians-University, Pettenkoferstr 8a, 80336 Munich, Germany*

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## 1. ABSTRACT

As other cytokine families, chemokines have multiple roles in local and systemic immune responses. In the kidney, the temporal and spatial expression of chemokines correlates with local renal damage. Chemokines play important roles in leukocyte trafficking and leukocyte activation, hence, blocking chemokines can effectively reduce renal leukocyte recruitment and subsequent renal damage. However, recent data indicate that blocking chemokine or chemokine receptor activity in renal disease may also exacerbate renal inflammation under certain conditions. An increasing amount of data indicate that additional roles of chemokines in the regulation of innate and adaptive immune responses may adversely affect the outcome of interventional studies. This review summarizes available *in vivo* studies on the blockade of chemokines and chemokine receptors in kidney diseases, with a special focus on the therapeutic potential and possible adverse effects of anti-chemokine strategies in renal inflammation.

## 2. INTRODUCTION

Independent of the initial injury chronic kidney disease (CKD) is commonly associated with a progressive loss of renal function. This process ultimately leads to end-stage renal failure, requiring continuous renal replacement therapy, i.e. dialysis or renal transplantation. Most common causes of CKD include diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, chronic tubulointerstitial disease, and polycystic kidney disease. With the incidence of diabetes, hypertension and associated renal injury increasing worldwide, the progression of CKD to end-stage renal disease is emerging as a major public health problem. The continuous decline of renal function is driven by a process of chronic inflammation and scarring involving both major compartments of the kidney, the glomeruli and the tubulointerstitial compartment. Features of this progressive fibrotic remodeling are activation of intrinsic renal cells, local infiltration of inflammatory leukocytes and accumulation of extracellular matrix, leading to glomerulosclerosis and tubulointerstitial fibrosis,

respectively (1-4). Chronic lesions in the glomerular compartment are characterized by macrophage infiltrates, activated intrinsic glomerular cells, i.e. glomerular endothelial cells, mesangial cells and podocytes, and glomerular matrix deposition. Chronic lesions in the tubulointerstitial compartment consist of mixed immune cell infiltrates (macrophages and T lymphocytes), proliferating myofibroblasts, and interstitial collagen deposits in the tubulointerstitial space (5).

What pathomechanisms cause these common histopathological abnormalities and how can we therapeutically interfere with the sequence of events? The local accumulation of leukocytes plays a central role in most pathways leading to progressive scarring of the kidney. In human biopsy studies of CKD tubulointerstitial leukocyte infiltrates correlate with the severity of renal failure (6, 7). Leukocyte depletion studies demonstrated the importance of immune cell infiltrates in fibrotic tissue remodeling (8). It is now widely accepted that effector functions of infiltrating renal leukocytes, most importantly macrophages and lymphocytes, directly and indirectly (via activation of adjacent renal cells) contribute to fibrotic tissue remodeling, both in immune-mediated (e.g. immune-complex glomerulonephritis) and nonimmune (e.g. diabetic nephropathy or hypertensive nephrosclerosis) renal diseases (9). As in other organs chemotactic molecules like chemokines are involved in the recruitment of leukocyte to the injured kidney. In this review the role of chemokines as mediators of CKD and potential pharmacological target are discussed. We will show that chemokines produced locally by intrinsic renal cells, resident and accumulating blood-borne leukocytes mediate an ongoing recruitment of immune cells into the two compartments of the kidney. Importantly, experimental blockade of chemokine-mediated leukocyte influx is associated with reduced renal scarring and improved kidney function in many studies. Despite some conflicting data from chemokine receptor-mutant mice and therapeutic studies with specific antagonists, interfering with chemokines and chemokine receptors may become a novel therapeutic avenue for retarding the progression of CKD.

## 3. PATHOMECHANISMS AND PROGRESSION OF CHRONIC KIDNEY DISEASE

Any type of kidney injury activates renal cells. Many forms of glomerular injury develop from predominant activation of a single glomerular cell type. For example, mesangial cells are predominantly activated in mesangioproliferative glomerulonephritis, like IgA nephropathy. Alternatively, endothelial cells are predominately activated in postinfectious glomerulonephritis, which can trigger local thrombus formation, focal necrosis or endothelial cell proliferation. Activated mesangial cells usually proliferate and produce extracellular matrix components including collagen type IV, laminin, and fibronectin leading to mesangioproliferative lesions and glomerulosclerosis (10). Podocyte activation can trigger a dedifferentiation process. The resulting podocyte detachment leads to proteinuria and focal glomerulosclerosis, as the complex secondary structure of podocytes is a crucial element of the glomerular filtration barrier (11). Activation of the parietal epithelia at the inner surface of Bowman's capsule will cause their proliferation and crescentic glomerular lesions. All types of activated renal cells secrete proinflammatory factors, including cytokines and chemokines. Proinflammatory cytokines like interleukin (IL)-6 activate adjacent cells while proinflammatory chemokines like CC chemokine ligand (CCL) 2/monocyte chemo attractant protein (MCP)-1 induce leukocyte infiltration to the site of injury (12). If the inflammatory process affects only one renal compartment, the glomerulus or the tubulointerstitium, the subsequent recruitment of leukocytes will be restricted to that compartment (13, 14). Infiltration, local activation and proliferation of leukocytes further enhance the local production of cytokines and chemokines, i.e. amplifying the initial signal provided by renal cells. Furthermore, neutrophils and macrophages generate radical oxygen species and lipid mediators that contribute to local tissue damage supporting a positive feedback mechanism. Macrophages are also the major source of growth factors such as fibroblast growth factor (FGF), transforming growth factor (TGF)-beta, tumor necrosis factor (TNF)-alpha, epithelial growth factor (EGF), and platelet-derived growth factor (PDGF) (15). In the tubulointerstitium fibroblast proliferation and secretion of extracellular matrix lead to widening of the interstitial space and renal fibrogenesis. Sources of the heterogeneous fibroblast population include proliferation of resident fibroblasts and myofibroblasts derived from tubular epithelial cells by a process described as epithelial-mesenchymal transition, two mechanisms that are induced by macrophage derived profibrotic cytokines such as FGF-2 (16). Moreover, circulating bone marrow-derived precursor cells, so-called fibrocytes, may infiltrate into diseased kidneys and differentiate into fibroblasts (17). Renal infiltration of fibrocytes is likely governed by locally secreted chemokines.

Proteinuria and secretion of proinflammatory mediators and growth factors stimulate tubular epithelial cells along the nephron to secrete proinflammatory and profibrotic cytokines, and chemokines in the tubulointerstitial compartment (12). Furthermore proinflammatory mediators secreted within the glomerular capillaries will reach the postglomerular peritubular vascular network, and hence, activate peritubular endothelial and tubular epithelial cells (18). All of these mechanisms may enhance interstitial mononuclear cell recruitment secondary to primary glomerular injury and thus expand the lesion from the glomerulus into the tubulointerstitium.

The continuous stimulation of intrinsic renal parenchymal cells by infiltrating leukocytes, proteinuria, and secreted cytokines results in ongoing synthesis of extracellular matrix components and irreversible structural damage. Obliteration of glomerular capillaries will destroy the entire nephron including downstream peritubular capillaries (19). The tubulointerstitial ischemia is considered to be an important factor for tubular cell apoptosis, necrosis, and, finally, tubular atrophy and

myofibroblast proliferation (5,20). The interstitial cell infiltrate itself together with the increasing amount of extracellular matrix leads to critical widening of the interstitial space. Thereby the distance of the remaining peritubular capillaries to their respective tubular segments increases, impairing oxygen diffusion as well as tubular reabsorbtive and excretory function (16). Thus, progressive glomerular and interstitial injury are tightly linked and aggravate each other by multiple mechanisms, including ischemia (21). As CKD progresses vascular rarification and diffuse scarring are associated with glomerulosclerosis and extensive tubular atrophy. The progressive loss of renal parenchyma and structural integrity finally results in end-stage renal disease, with shrunken kidneys and the clinical signs and symptoms of uremia.

## 4. IMMUNOSUPPRESSIVE VERSUS ANTI-INFLAMMATORY TREATMENT STRATEGIES FOR CHRONIC KIDNEY DISEASE

Current treatments of CKD include blood pressure control, angiotensin inhibition, and avoiding the exposure to nephrotoxins such as contrast media, aminoglycosid antibiotics or nonsteroidal antiinflammatory drugs. Immunosuppressive therapies with steroids and cytotoxic agents are rarely used because evidence for their efficacy on CKD progression is only available for a few types of CKD. For example, immunosuppression with cytotoxic drugs is effective in crescentic glomerulonephritis, diffuse proliferative lupus nephritis, anti-GBM glomerulonephritis, and renal vasculitis (22-25). However, the most common types of CKD are diabetic nephropathy and vascular nephropathy. Due to the presumed "non-inflammatory" pathogenesis of these disease entities immunosuppressive therapies have not yet been considered. In addition, chronic forms of glomerulonephritis like IgA nephropathy represent another common cause for CKD. IgA nephropathy is an immune complex-mediated disease. Hence, immunosuppression was anticipated to potentially control disease progression. However, the controversial results of clinical trials do not support immunosuppressive treatment of most patients (26, 27). In addition, the side effects of steroids or cytotoxic agents limit their use in CKD. For example, steroids increase blood pressure, are prodiabetic, and their chronic use is associated with significant complications in eyes, bone, and skin. Alkylating agents and other cytotoxic immunosuppressants are associated with myelosuppression and secondary malignancies. These problems are related to the unspecific effects of steroids and cytotoxic drugs which interfere with many physiological pathways unrelated to the target organ. In contrast to these nonspecific immunosuppressive therapies with their associated morbidity (and mortality) a selective targeting of inflammatory effector systems in CKD could potentially lead to more disease-specific therapeutic approaches with higher efficacy and less systemic side effects. To reduce renal inflammation and scarring a variety of immunologic effector systems have been targeted in experimental models including complement, cytokines/chemokines, adhesion molecules, mediators of cellular proliferation and by selective depletion of single immune cell subsets (22). Some of these more selective anti-inflammatory therapies have demonstrated to effectively control chronic inflammation in the absence of major side effects in human diseases, e.g. rheumatoid arthritis (28, 29). The following paragraphs will describe the rationale for blocking the chemokine network in CKD. Current experimental data will be summarized that provide evidence for chemokine and chemokine receptors being a potential target for a selective anti-inflammatory therapy in CKD.

## 5. EXPERIMENTAL EVIDENCE FOR CHEMOKINES AND CHEMOKINE RECEPTORS MEDIATING GLOMERULAR AND INTERSTITIAL LEUKOCYTE INFLUX AND INJURY

What experimental evidence supports a functional role for chemokines and chemokine receptors (CR) as mediators of renal disease? Their involvement in the pathogenesis of glomerular and interstitial injury has been demonstrated in various animal models by blocking chemokine activity with neutralizing antibodies, CR antagonists, and targeted disruption of chemokine as well as CR genes.

### 5.1. Glomerulonephritis

In the rat, neutralizing antibodies to CXC chemokine ligands (CXCL), including CXCL1/CINC and CXCL2/3/MIP-2 reduced glomerular neutrophil infiltration and proteinuria during the acute phase of nephrotoxic serum nephritis (30), a widely used model for immune complex-mediated glomerulonephritis. Blockade of the CXC chemokine receptor CXCR2, which binds multiple CXC chemokines, with the peptide analog GRO (growth regulated oncogene)-alpha$_{8-73}$ demonstrated a role of CXCR2 in mediating glomerular monocyte recruitment in the same model (30). Inhibition of multiple CC and CXC chemokine receptors by vMIP-II, a viral broad chemokine antagonist encoded by human herpesvirus 8, also decreased proteinuria and glomerular infiltration of macrophages and $CD8^+$ T cells in rats (31). In a rat model of thrombotic microangiopathy with glomerular and tubulointerstitial injury Panzer *et al.* noted an increased tubulointerstitial expression of the T-cell chemokine CXCL10/interferon-inducible protein (IP)-10. Blocking CXCL10/IP-10 with an antagonistic antibody largely prevented the interstitial T cell infiltrate in this model, and improved renal function (32). The CXCL10 receptor CXCR3 is predominantly expressed by Th1-type effector T cells. Consistently, the same group demonstrated a reduced renal T cell infiltrate in CXCR3-deficient mice subjected to accelerated nephrotoxic serum nephritis. The decreased number of infiltrating T cells correlated with reduced glomerular and interstitial renal injury in the CXCR3 knockout mice, being consistent with the Th1-dependency of this model (33).

Many members of the CC chemokine subfamily mediate proinflammatory effects. Thus, several studies focused on the role of CC chemokines in mediating renal leukocyte accumulation and tissue injury. Neutralizing antibodies to the CC chemokine CCL2/MCP-1, a major monocyte/macrophage chemo attractant, blocked glomerular infiltration of macrophages in rat anti-Thy-1.1

nephritis, a model of mesangioproliferative glomerulonephritis (34). In rats with nephrotoxic serum nephritis selective blockade of CCL2/MCP-1 expression in tubular epithelial cells by an antisense approach resulted in reduced tubulointerstitial damage (35). Treatment with anti-CCL2/MCP-1 antibodies also reduced glomerular macrophage influx and proteinuria in rat nephrotoxic serum nephritis (36, 37), and abrogated glomerular leukocyte infiltration, proteinuria, crescent formation and interstitial collagen deposition in murine nephrotoxic nephritis (38). However, when CCL2/MCP-1-deficient knockout mice were subjected to nephrotoxic serum nephritis, glomerular injury and proteinuria were comparable to wild-type mice, and only the tubulointerstitial injury was reduced (39). The discrepant results to the interventional study in the same disease model may be due to differential effects of CCL2/MCP-1 deficiency versus antibody blockade on mounting the nephritogenic immune response, or due to compensatory chemokine and chemokine receptor systems that develop in genetically deficient mice. Beneficial effects of two CCL5/regulated on activation, normal T-cell expressed and secreted (RANTES) analogs, amino-oxypentane (AOP)-RANTES and Met-RANTES, which block binding of CCL5/RANTES to its receptors CCR1, CCR3, and CCR5, have been demonstrated in rodent studies. AOP-RANTES inhibited glomerular macrophage infiltration in an anti-Thy 1.1 nephritis model (40). Met-RANTES reduced glomerular and interstitial leukocyte infiltration as well as proteinuria in murine nephrotoxic serum nephritis, although glomerular crescent formation and renal collagen deposition was not affected (38). Moreover, a preliminary report noted that nephrotoxic serum nephritis was not ameliorated in CCR5 deficient mice, indicating that CCL5/RANTES may mediate renal leukocyte infiltration and injury through receptors other than CCR5 (41).

Finally, the $CX_3C$ chemokine $CX_3CL1$/fractalkine apparently has a functional role in mediating glomerular injury. Blocking its receptor $CX_3CR1$ with a neutralizing antibody improved renal function and prevented crescentic glomerulonephritis in rat nephrotoxic nephritis (42).

### 5.2. Interstitial nephritis

Several functional studies demonstrated a role of chemokines and chemokine receptors in directly mediating interstitial leukocyte infiltration and subsequent interstitial nephritis and fibrosis. Blockade of the chemokine receptor CCR1 with the small molecule antagonist BX471 effectively reduced interstitial infiltration of mononuclear leukocytes (i.e. macrophages and T lymphocytes) in a model of primary interstitial injury induced by unilateral ureteral obstruction (UUO) (43). Moreover, markers of renal fibrosis, such as interstitial fibroblasts, interstitial volume, collagen I mRNA and protein expression 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 of the model, indicating that late onset of CCR1 blockade is still effective (43). Similar results were obtained when mice genetically deficient for CCR1 were investigated (44). In the same model Kitagawa *et al.* demonstrated a reduction of interstitial leukocyte infiltrates, most of which were F4/80-positive cells, in CCR2 knockout mice and after treatment with the CCR2 antagonists propagermanium and RS-504393 (45). Consistently, interstitial leukocyte infiltration was also reduced in UUO mice transfected with the gene construct 7ND. This construct encodes a truncated, antagonistic CCL2/MCP-1 protein, which blocks activation of CCR2 by all of its endogenous ligands including CCL2, CCL7/MCP-3, CCL8/MCP-2, and CCL13/MCP-4 (46). 7ND gene therapy also reduced interstitial macrophage infiltration in a rat model of protein overload proteinuria (47). Importantly, all of these studies demonstrated that chemokine and chemokine receptor antagonism not only reduced interstitial leukocyte infiltrates, but also decreased tubulointerstitial pathology, most importantly interstitial matrix accumulation and fibrosis. As these interstitial changes are key histopathologic features of CKD that closely correlate with the progressive decline in renal function, blocking renal chemokine activity may be a promising new therapeutic avenue to halt the progression of CKD.

## 6. EXPERIMENTAL EVIDENCE FOR A ROLE OF CHEMOKINES AND CHEMOKINE RECEPTORS IN THE PROGRESSION OF CHRONIC KIDNEY DISEASE

As discussed above, many chemokines and CR have been identified as mediators of renal leukocyte infiltration and injury, both in the glomerular and tubulointerstitial compartment. Do we also have evidence that the chemokine system mediates progression of chronic kidney disease? Indeed, this concept is supported by functional studies in models of chronic progressive renal disease such as focal segmental glomerulosclerosis (FSGS), lupus nephritis, diabetic nephropathy and Alport syndrome.

### 6.1. Focal segmental glomerulosclerosis

As discussed before, proteinuria is an important mediator of progression in chronic renal disease. Importantly, unselective proteinuria can induce chemokine expression in renal tubular cells (18), thereby serving as a major factor for tubulointerstitial inflammation. Thus, we recently investigated whether blockade of the chemokine receptor CCR1 would be able to improve interstitial inflammation and fibrosis in the presence of nephrotic range proteinuria caused by FSGS. FSGS can be induced in BALB/c mice by intravenous injection of adriamycin leading to massive proteinuria, glomerular sclerosis and tubulointerstitial fibrosis within 6 weeks (adriamycin nephropathy) (48, 49). When CCR1 was blocked with the small molecule antagonist BX471 given from week 2 to 6 (i.e. after glomerular injury and proteinuria had developed), the amount of interstitial macrophages and T cells as well as markers of renal fibrosis including interstitial fibroblast accumulation and interstitial volume expansion was reduced (50). Interestingly, BX471 did not affect glomerular pathology in adriamycin nephropathy. These findings demonstrate that therapeutic CCR1 blockade effectively reduces interstitial pathology in the presence of heavy proteinuria. In a rat model of adriamycin nephropathy Wu *et al.* demonstrated that DNA vaccination

with naked DNA encoding CCL2 and CCL5 ameliorated the progression of renal disease. Treated rats developed less proteinuria, had better renal function, less glomerular sclerosis and interstitial infiltrates (51). Thus, chemokine DNA vaccination reduced both glomerular and tubulointerstitial injury in this model of chronic proteinuric renal disease, potentially by induction of neutralizing autoantibodies against CCL2/MCP-1 and CCL5/ RANTES (51). Interestingly, DNA vaccination with a modified CCL2 DNA containing a foreign T helper cell epitope (tetanus toxoid epitope P30) could further boost its immunogenicity by eliciting a strong self-specific CCL2/MCP-1 autoantibody production, as well as an interferon (IFN)-gamma-producing T cellular response. This was associated with a more effective blockade of glomerular and interstitial macrophage recruitment and protection against renal injury, compared to the unmodified DNA vaccine (52).

**6.2. Lupus nephritis**

Lupus nephritis is a classic form of immune complex-mediated nephritis, and progressive renal insufficiency a leading cause of mortality in patients with systemic lupus erythematosus (53). Recent work identified chemokines and chemokine receptors as important mediators of renal inflammation and progressive injury in murine models of lupus nephritis (reviewed in ref. 54). We studied effects of a therapeutic blockade of CCR1 in progressive lupus-like immune complex glomerulonephritis that spontaneously develops in MRL/lpr mice. Treatment with the CCR1 antagonist BX471 initiated late during the course of disease (weeks 20 to 24 of age) improved blood urea nitrogen levels and reduced the amount of interstitial macrophage and T cell infiltrates (55). 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. However, CCR1 blockade did not reduce glomerular macrophage recruitment. Consistently, it was ineffective in attenuating glomerular pathology and proteinuria in MRL/lpr mice, a finding similar to the results obtained in the adriamycin nephropathy model (50).

In contrast, the CCL2/MCP-1-CCR2 axis apparently mediates glomerular leukocyte infiltration and injury in lupus nephritis. MRL/lpr mice genetically deficient in CCL2/MCP-1 lived longer than CCL2/MCP-1 intact MRL/lpr controls, and were protected from the loss of renal function (56). Infiltration of glomerular macrophages, but not T cells was reduced in CCL2/MCP-1-deficient mice. This was associated with less proteinuria and less glomerular injury, i.e. glomerular hypercellularity, glomerulosclerosis, and crescent formation. In the interstitium accumulation of both macrophages and T cells was reduced, and this was accompanied by a decrease in tubulointerstitial injury including tubular atrophy and apoptosis. The largest decline in renal leukocyte infiltrates occurred at the sites of the most abundant CCL2/MCP-1 expression, i.e. the tubulointerstitial compartment (56). A non-redundant role for the CCL2/MCP-1-CCR2 axis in lupus nephritis was confirmed when CCR2-deficient MRL/lpr mice were studied (57). CCR2-deficient animals survived significantly longer than MRL/lpr wild type controls. CCR2 deficiency reduced lymphadenopathy, and glomerular and tubulointerstitial lesion scores in MRL/lpr kidneys. This was accompanied by a reduced infiltration of macrophages and T cells both into the glomerular and tubulointerstitial compartment. Together, the data obtained in CCL2/MCP-1 and CCR2-deficient mice indicate an important role of this pathway in mediating both glomerular and interstitial renal disease in lupus.

These findings were validated by several interventional studies utilizing CCL2/MCP-1-CCR2 antagonists. Hasegawa *et al*. showed that an NH(2)-terminal-truncated CCL2 analogue can block glomerular as well as interstitial macrophage and T cell recruitment in MRL/lpr mice with lupus nephritis (58). They initiated an 8 weeks course of treatment with this CCL2 analog in 7 and 12 week old mice, thereby mimicking treatment in pre-nephritic and early nephritic MRL/lpr mice. Both protocols resulted in a delay of renal damage. Glomerular hypercellularity, glomerulosclerosis, crescent formation and vasculitis were reduced compared with control mice. CCL2/MCP-1 antagonism from week 12 to 20 resulted in a markedly diminished infiltration of macrophages and T cells into glomeruli, the interstitium, and perivascular areas [49]. Subsequent studies confirmed these findings by using gene transfer of a plasmid transfection vector encoding the 7ND truncated CCL2/MCP-1 analog, which acts as a CCR2 receptor blocker, into skeletal muscles of 16 week old MRL/lpr mice (59). Treated MRL/lpr mice showed a survival benefit and ameliorated glomerulonephritis with reduced crescent formation. Both glomerular and interstitial macrophages were reduced. However, in this study CCL2/MCP-1 antagonism did not influence vasculitic lesions (59). Additional studies from the same group defined that a delayed 7ND gene transfer reduced the local Th1-immune response in MRL/lpr kidneys, but did not affect a presumed role of CCL2/MCP-1 in helper T cell polarization (60). This was evident by unaltered production of IFN-gamma and Il-12 in splenocytes, whereas glomerular expression of Il-12 and interstitial expression of IFN-gamma and Il-12 were significantly reduced (60). Our own group recently demonstrated that administration of an l-enantiomeric RNA oligonucleotide, a so-called spiegelmer, that binds murine CCL2/MCP-1 with high affinity and neutralizes its action *in vivo*, ameliorated lupus nephritis (61). When MRL/lpr mice were treated with a polyethylene glycol form of the spiegelmer from weeks 14 to 24 of age, these mice showed prolonged survival associated with a robust improvement of lupus nephritis, including less glomerular and interstitial injury and reduced renal macrophage and T cell infiltrates (61).

Inoue *et al*. have evaluated blockade of the $CX_3C$ chemokine $CX_3CL1$/fractalkine with a truncated $CX_3CL1$/fractalkine analog in MRL/lpr mice (62). When the $CX_3CL1$/fractalkine analog was given to 8 week old MRL/lpr mice for another 8 weeks glomerular and tubulointerstitial damage improved, which was associated with a reduced number of $CX_3CR1$ positive monocytes in the glomerular and interstitial compartment. Interestingly, the beneficial effect of the $CX_3CL1$/fractalkine analog was

restricted to autoimmune tissue injury in the kidney, in contrast to lungs and salivary glands, which might be attributed to the expression of $CX_3CL1$/fractalkine on glomerular and peritubular endothelial cells (62).

Homeostatic chemokines have different functions than the inflammatory chemokines discussed above. However, blocking homeostatic chemokines may also have beneficial effects on the progression of inflammatory renal disease such as lupus nephritis, for example through interfering with systemic immune responses and the generation of effector leukocytes. For example, the CXC chemokine CXCL12/SDF-1 has important functions in B lymphocyte and monocyte development. The administration of a CXCL12/stromal cell-derived factor (SDF)-1 antagonist to lupus-prone New Zealand black/New Zealand white (NZB/NZW) F1 mice with established lupus nephritis reduced DNA autoantibody production, glomerular immune complex deposits, proteinuria, and renal injury (63).

### 6.3. Diabetic nephropathy

Diabetic nephropathy is the most common type of chronic kidney disease progressing to terminal renal failure. Uninephrectomized db/db mice develop chronic diabetic nephropathy within 6 months of age. In this model, oral treatment with the CCR1 antagonist BL5923 from months 5 to 6 of life reduced the numbers of interstitial macrophages (64). This was associated with reduced numbers of proliferating tubulointerstitial cells, tubular atrophy, and interstitial fibrosis. As shown previously in other murine disease models, CCR1 blockade did not affect glomerular pathology and proteinuria in db/db mice (64). Thus, CCR1 antagonism could improve tubulointerstitial, but not glomerular disease in late-stage diabetic nephropathy.

As interference with CCL2/MCP-1 and CCR2 had the most beneficial effects in terms of glomerular and tubulointerstitial pathology in several renal disease models, blockade of the CCL2/MCP-1-CCR2 axis might be a valuable therapeutic approach also in diabetic nephropathy. Two studies have been published that support a functional role for CCL2/MCP-1 in diabetic renal injury. Chow *et al.* showed that CCL2/MCP-1 promotes the development of diabetic nephropathy in streptozotocin-treated mice (65). Diabetic CCL2/MCP-1-deficient mice developed less albuminuria and had less elevated plasma creatinine levels at 18 weeks of age. This was associated with reductions in glomerular and interstitial macrophage accumulation, glomerular injury and interstitial fibrosis (65). The same authors investigated the role of CCL2/MCP-1 in a more chronic, type 2 diabetic nephropathy model developing in db/db mice. Again, kidney macrophage accumulation and the progression of diabetic renal injury, i.e. albuminuria, glomerular pathology and tubulointerstitial fibrosis were substantially reduced in CCL2/MCP-1-deficient db/db mice at 32 weeks of age (66). Consistently, a recent interventional study could demonstrate that inhibition of the CCL2/MCP-1-CCR2 pathway ameliorated the development of diabetic nephropathy (67). When mice were treated with the CCR2 antagonist propagermanium or were transfected with plasmids expressing the inhibitory CCL2/MCP-1 analog 7ND, glomerular macrophage infiltration and mesangial matrix expansion as well as glomerular expression of collagen IV and TGF-beta 1 were reduced (67). Although this study provides no data on functional outcomes, i.e. serum creatinine levels or albuminuria, these data indicate that pharmacological targeting of the CCL2/MCP-1-CCR2 axis could halt progression of diabetic nephropathy.

### 6.4. Glomerulosclerosis in Alport nephropathy

Alport syndrome caused by mutations in the procollagen IV genes is a hereditary glomerulopathy leading to glomerulosclerosis with subsequent interstitial fibrosis and progression to end-stage renal disease (68). Collagen IV A3-deficent mice develop renal lesions comparable to human Alport disease, and recent studies have documented a role for interstitial macrophages mediating disease progression in these mice (69). When collagen IV A3-deficent mice were treated with the CCR1 antagonist BX471 from 6 weeks of age for 4 weeks, interstitial macrophage accumulation decreased significantly (70). BX471 treatment was associated with less apoptotic tubular epithelial cells, tubular atrophy, interstitial fibrosis, and less globally sclerotic glomeruli. In contrast, BX471-treated mice showed a higher density of peritubular capillaries. Importantly, CCR1 blockade significantly increased the mean survival of collagen IV A3-deficent mice from 69 to 86 days (70). Thus, blocking CCR1-mediated interstitial macrophage recruitment can maintain peritubular microvasculature and prevent tubulointerstitial fibrosis, two major hallmarks of disease progression in CKD.

## 7. COMPARTMENT-SPECIFIC FUNCTIONS OF CHEMOKINES AND CHEMOKINE RECEPTORS IN THE KIDNEY

Renal expression of proinflammatory chemokines like CCL2/MCP-1 and CCL5/RANTES generally is restricted to the injured compartment of the kidney. In acute glomerulonephritis chemokines are exclusively produced within glomeruli (13), whereas in primary tubulointerstitial diseases like obstructive nephropathy, chemokine expression is confined to tubular epithelial cells and interstitial infiltrates (14). When progression of a glomerular disease leads to secondary tubulointerstitial damage, chemokines are produced in both compartments (71). Moreover, the spatial expression of chemokines correlates with local accumulation of chemokine receptor-positive leukocytes at sites of renal damage (13-14,71). Thus, interventions to target chemokine-mediated inflammatory responses may exert its effects specifically in the injured compartment of the kidney.

However, further points must be considered that have important implications for chemokine targeting therapies in renal disease. First, a given chemokine may be expressed only in a single renal compartment, and may attract only a certain leukocyte subset expressing the respective chemokine receptor. For example, CXCL10/IP-

10 expression was exclusively found in the tubulointerstitium of rats with thrombotic microangiopathy leading to glomerular and tubulointerstitial injury (32). Consistently, CXCL10/IP-10 blockade selectively reduced interstitial infiltrates of T cells known to express the CXCL10/IP-10 receptor CXCR3, and improved renal function. In contrast, renal infiltration of macrophages and albuminuria (reflecting the degree of glomerular structural damage) was not affected. In progressive renal disease it has long been recognized that T cells preferentially accumulate in the tubulointerstitium, but not in glomeruli. This study not only identified CXCL10/IP-10 as a major candidate for the mechanism that attracts T cells specifically into the interstitium, but not glomeruli. The data also indicate that targeting CXCL10/IP-10 may be a promising approach to reverse interstitial renal disease, but likely is not effective in ameliorating glomerular injury. Interestingly, CXCR3-deficiency did ameliorate both glomerular and interstitial disease in a model of nephrotoxic serum nephritis that is T cell dependent (33). As humoral and cellular nephritogenic immune responses were intact in CXCR3-deficient mice, other chemokines than CXCL10/IP-10 may mediate CXCR3-dependent glomerular leukocyte influx and injury.

Secondly, chemokine receptors may have compartment-specific roles in renal leukocyte recruitment, although expression of respective chemokine ligands is not restricted to single compartments. As discussed earlier we showed that the CCR1 antagonist BX471 could effectively reduce interstitial macrophage and T cell recruitment as well as renal fibrosis in obstructive nephropathy (43). Blockade of CCR1 in adriamycin-induced FSGS with secondary interstitial nephritis also reduced interstitial leukocyte accumulation and injury, whereas the extent of proteinuria and glomerular sclerosis was not affected (50). This compartment-specific function of CCR1 was confirmed in models of lupus nephritis, diabetic nephropathy, and Alport syndrome, where application of CCR1 antagonists reduced macrophage and T cell infiltration into the interstitium, but not into glomeruli. This was paralleled with ameliorated interstitial, but not glomerular injury (55,64,70). In MRL/lpr mice with lupus nephritis, adoptive cell transfer studies with labeled leukocytes clearly demonstrated that CCR1, while critical for interstitial leukocyte recruitment, was not required for their infiltration into the glomerular compartment (55). In contrast, we could show that CCR5, which has overlapping ligand specificity with CCR1, mediates glomerular, but not interstitial macrophage recruitment (44). Given the fact that CCR1 and CCR5 are expressed on circulating monocytes and T cells, other factors appear to determine their selective roles for recruitment in different renal microvascular beds. For example, differential expression of endothelial molecules needed for chemokine binding and presentation, e.g. proteoglycans or the Duffy antigen receptor for chemokines (DARC), may account for the compartment-specific activation of chemokine receptors. Importantly, these data indicate that targeting certain chemokine receptors like CCR1 may be beneficial in interstitial, but not glomerular disease. In contrast, many of the interventional studies discussed above proved a functional role of the CCL2/MCP-1-CCR2 axis in mediating both glomerular and interstitial renal injury.

## 8. CONFLICTING DATA ON THE ROLE OF CHEMOKINES AND CHEMOKINE RECEPTORS IN RENAL DISEASE

Targeting chemokines not only interferes with local recruitment and activation of inflammatory cells. Chemokines also have crucial roles in leukocyte development and maturation, and thus in orchestrating effector mechanisms of the innate and adaptive immune system. Therefore, chemokine antagonism may potentially interfere with important regulatory functions of the immune system that suppress (auto-) immune responses. This may aggravate renal disease, rather than reduce disease activity, especially in primarily immune-mediated nephropathies. For example, CCR1-deficient mice developed more severe glomerular pathology, greater proteinuria and an increased renal infiltration of macrophages and T cells during nephrotoxic serum nephritis (72). Interestingly, enhanced Th-1 responses were noted in these mice, suggesting an altered systemic immune response in the effector phase as a potential mechanism of exacerbation (72).

Moreover, in mice lacking CCR2 proteinuria and glomerular pathology of nephrotoxic serum nephritis was worse throughout the study period, despite reduced glomerular macrophage infiltration at early time points (73). Potential mechanisms of this aggravation were not explored in this study. However, CCL2/MCP-1-deficient mice have diminished cellular and humoral Th-2 responses (74), which could exacerbate a Th1-driven disease model like nephrotoxic serum nephritis. Moreover, CCL2/MCP-1-CCR2 signaling may be important to recruit and activate CCR2 positive regulatory T cells, which are essential to down-regulate adaptive immune responses. Indeed, in a model of collagen-induced arthritis, late onset blockade of CCR2 with a monoclonal antibody markedly aggravated arthritis and increased the humoral immunity against collagen. Blockade of CCR2-positive regulatory T cells was implicated in this finding (75).

We have shown that the CCL5/RANTES antagonists Met-RANTES and AOP-RANTES both unexpectedly aggravated glomerular damage and proteinuria in acute immune-complex glomerulonephritis in mice, despite reduction of glomerular macrophage infiltration (76). This was associated with an enhanced proinflammatory state of the macrophages present in the glomerulus, indicated by increased iNOS expression and reduced uptake of apoptotic cells (76). Thus, the CCL5/RANTES antagonists altered the phenotype of infiltrating cells during renal inflammation, either by selectively blocking CCL5/RANTES-dependent infiltration of macrophages with a “beneficial”, anti-inflammatory phenotype or by agonistic effects on CCL5/RANTES receptors, which have been described for these analogs (77).

## 9. VALIDATING POTENTIAL CHEMOKINE AND CHEMOKINE RECEPTORS TARGETS FOR THERAPEUTIC APPLICATIONS

Interventional studies are required to validate chemokines and chemokine receptors for potential therapeutic use. Several points have to be taken into consideration when such studies are designed to yield valid results. Specific agonists or antagonists are ideally suited to test the functional significance of a potential chemokine target, as they can be applied following the induction of disease models. Because of species-specific structures and functions of some chemokines detailed information about the compounds` crossreactivity with the target in the species of interest are necessary. Furthermore, due to the high degree of homology between the chemokines and the chemokine receptors any effort must be undertaken to assure the specificity of the compound. The rationale for dosing the animal must be evident from appropriate pharmacodynamic and pharmacokinetic assays. Sufficient compound levels at the organ site of interest depends on a variety of factors, including adsorption, distribution, metabolism, and excretion of the compound. In kidney disease models renal dysfunction may significantly alter the pharmacokinetics of the compound which may require pharmacokinetic studies at various stages of renal dysfunction. Inbred mouse or rat strains remain the preferred tool for *in vivo* validation study due to low genetic variability. However, many disease models depend on a defined (outbred) strain of mice or rats.

The use of disease models that require months to develop may require an unacceptable number of injections or manipulations. Hence, short term models are usually preferred but these may not always mirror the human disease state, especially in CKD. For example, diabetic nephropathy needs up to 15 years to develop in humans. Hence, it comes to no surprise that advanced diabetic nephropathy does not develop before 8 months of age in obese db/db mice. The benefit from such study protocols need to be balanced against the availability of the compound to be tested or the feasibility of drug administration. Certain frequently used disease models in target identification studies are less useful for target validation. For example, injection of a Thy-1 antibody induces mesangiolysis and a compensatory mesangioproliferative glomerular lesion in rats (78). However, the pathomechanisms of this anti-Thy-1-nephritis lacks an equivalent human disease.

Can knock-out mice be used for validating chemokine targets? Data from conventional gene-deficient mice do not necessarily predict a potential therapeutic significance of the target chemokine or chemokine receptor. In fact, lack of the gene product may affect the induction of disease models. For example, models of antigen-specific immunity require dendritic cell activation and migration to lymphnodes in its early phase. Lack of chemokine receptor CCR7 inhibits this process and models involving antigen-specific immunity will not develop properly (79). The inconsistent autoimmune phenotype of MRL/lpr mice which either lack Toll-like receptor 9 or are treated with a respective receptor antagonist is one example (80-81). By contrast, a single cell type can functionally be silenced on demand using conditional knock-out mice by exposing the animal to a "silencer drug" (8).

The same applies to target validation studies with drug-like compounds. In a clinical setting patients present with a clinical manifestation of disease before treatment is considered. Accordingly, drug administration to the animal should not be initiated before the disease model has been established. For example, blockade of chemokine receptor CCR2 has opposite effects on collagen-induced arthritis, when initiated before or after immunization with collagen (75).

Together, when the criteria for *in vivo* target validation studies are applied to the chemokine field only a limited number of reports meet the mentioned requirements and may be useful to predict a therapeutic outcome of a chemokine-based intervention in disease.

## 10. PERSPECTIVE – DATA NEEDED BEFORE APPROACHING CLINICAL TRIALS

The current experimental data suggest that chemokine or chemokine receptor blockade might be effective for the treatment of human CKD. However, before approaching clinical trials few more questions should be addressed. First, blocking single chemokines or chemokine receptors mostly reduced glomerular or interstitial leukocytes by no more than 50%. Hence, blocking multiple rather than single chemokines or chemokine receptors may be more efficient in blocking renal leukocyte recruitment and in preventing disease progression. To identify potential target combinations more data are required on compartment-specificity and non-redundant functions of single chemokines in the kidney. Hence, the future of chemokine-based anti-inflammatory drugs in the field of nephrology will depend on the collaboration of basic scientists, clinical investigators, and industrial partners that share the motivation to develop novel treatments for patients with CKD.

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**Abbreviations:** CKD: chronic kidney disease; IL: interleukin; CCL: CC chemokine ligand; CR: chemokine receptor; MCP: monocyte chemoatractant protein; FGF: fibroblast growth factor; TGF: transforming growth factor; TNF: tumor necrosis factor; EGF: epithelial growth factor; PDGF: platelet-derived growth factor; CXCL: CXC chemokine ligand; MIP: macrophage inflammatory protein; CINC: cytokine-induced neutrophil chemo attractant; GRO: growth regulated oncogene; vMIP: viral macrophage inflammatory protein; IP: interferon-inducible protein; RANTES: regulated on activation, normal T-cell expressed and secreted; UUO: unilateral ureteral obstruction; FSGS: focal segmental sclerosis; DNA: desoxyribonucleic acid; IFN: interferone; SDF: stromal cell-derived factor; NZB/NZW: New Zealand black/New Zealand white; iNOS: inducible nitric-oxidase synthase; DARC: Duffy antigen receptor for chemokines;

**Key Words:** Chemokines, Kidney Disease, Glomerulonephritis, Glomerulosclerosis, Inflammation, Review

**Send correspondence to:** Volker Vielhauer, Medizinische Poliklinik, Klinikum der Universitat Munchen - Campus Innenstadt, Ludwig-Maximilians-University, Pettenkoferstr. 8a, 80336 Munich, Germany, Tel: 49-89-2180-75007, Fax: 49-89- 2180-75860, E-mail: volker.vielhauer@med.uni-muenchen.de

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