Arch Immunol Ther Exp, 2004, 52, 164–172
PL ISSN 0004-069X

WWW.AITE–ONLINE.ORG
Review

**Received:** 2003.12.24
**Accepted:** 2004.02.26
**Published:** 2004.06.15

# Targeting the chemokine network in renal inflammation

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**Source of support:** grants from the German Academic Exchange Service and the Board of the German Society of Nephrology to Dr. V. Eis, the Deutsche Forschungsgemeinschaft (LU 612/4-1), the German Kidney Foundation and Wilhelm Sander Foundation to Dr. H. J. Anders.

## Summary

Chemokines and their receptors are involved in the pathogenesis of renal diseases**.** They mediate leukocyte recruitment and activation during initiation as well as progression of renal inflammation. Infiltrating leukocyte subpopulations contribute to renal damage by releasing inflammatory and profibrotic cytokines. All intrinsic renal cells are capable of chemokine secretion on stimulation *in vitro*. Expression of inflammatory chemokines correlates with renal damage and local accumulation of chemokine receptor-bearing leukocytes in a variety of animal models of renal diseases as well as in human biopsy studies. Chemokines and their respective receptors could represent new targets for therapeutic intervention in renal inflammatory disease states that often tend to progress to end-stage renal disease. This article summarizes the present data on the role of chemokines and their receptors in renal inflammation with special emphasis on our efforts to identify the chemokine receptors CCR1 and CCR2 as promising targets for therapeutic intervention.

**Key words:** **chemokines • inflammation • kidney disease • fibrosis**

**Full-text PDF:** http://www.aite-online/pdf/vol_52/no_3/5535.pdf

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## INTRODUCTION

Inflammatory diseases of the kidney that progress to end-stage renal disease are characterized by tubular damage and atrophy, massive proliferation of fibroblasts, excessive matrix deposition, as well as the accumulation of interstitial leukocytes. Infiltrating leukocytes are a major source of proinflammatory and profibrotic cytokines and are therefore critical in mediating fibroblast proliferation, differentiation into myofibroblasts, matrix production, and tubular damage[15]. The concerted interaction of chemokines and adhesion molecules has been shown to play an important role in leukocyte recruitment, activation, and effector function[33, 43, 45]. Thus, such molecules directing circulating leukocytes into the interstitial space may provide new therapeutic targets in progressive renal disease. In view of the hypothesis that blockade of chemokine-driven leukocyte infiltration may be a valuable approach for the treatment of progressive renal disease, this overview focuses on the identification of potential targets for therapies in the field of chemokine biology.

## CHEMOKINES

Chemokines are a large family of low-molecular--weight cytokines that induce migration of leukocytes and modulate multiple functions of immune and non-immune cells[67]. The official nomenclature used to describe individual chemokines is based upon the four-cysteine motif in their primary amino acid sequence, for example, CC, CXC, CX3C, and C chemokines (Table 1)[67]. The new systematic nomenclature classifies chemokines by the above criteria but also by their binding characteristics as ligands (L), such as CL, CCL, CXCL and CX3CL. For example, CX3CL is the only known CX3C chemokine and has a single receptor, CX3CR. CX3CL is tethered directly to the cell membrane via a mucin stalk and combines the function of a chemokine and adhesion molecule[35]. Accordingly, the C chemokine XCL1 binds to XCR1[43]. CXC chemokines can be further subclassified by structure into $ELR^+$ and $ELR^-$ chemokines based on the presence or absence of the tripeptide motif glutamic acid-leucine-arginine (ELR) N-terminal to the first cysteine. The ELR-CXC chemokines generally act as neutrophil chemoattractants and promotors of angiogenesis. On the other hand, CXC chemokines without the ELR motif bind to different receptors on lymphocytes and inhibit angiogenesis.

Chemokines can be further classified according to their predominant function and expression pattern. For example, chemokines such as CCL2, CCL5, and CXCL10 that are involved in the recruitment of leukocytes to sites of tissue injury are classified as proinflammatory chemokines. Such chemokines are up-regulated by proinflammatory stimuli and orchestrate innate and adaptive immune responses, such as

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

| Ligand | | Receptors | Ligand | | Receptors |
|---|---|---|---|---|---|
| **CXCL** | | | **CCL** | | |
| CXCL1 | Groα | CXCR1,2 | CCL1 | I-309 | CCR8 |
| CXCL2 | Groβ | | CCL2 | MCP-1 | CCR2 |
| CXCL3 | Groγ | | CCL3 | MIP-1α | CCR1, 5 |
| CXCL4 | PF4 | CXCR1,2 | CCL4 | MIP-1β | 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 |
| **XCL** | | | CCL21 | SLC/6Ckine | CCR7 |
| XCL1 | Lymphotactin | XCR1 | CCL22 | MDC | CCR4 |
| XCL2 | SCM-1β | | CCL23 | MPIF-1 | CCR1 |
| | | | CCL24 | Eotaxin-2 | CCR3 |
| | | | CCL25 | TECK | CCR9 |
| **CX3CL** | | | CCL26 | Eotaxin-3 | CCR3 |
| CX3CL1 | Fractalkine | CX3CR1 | CCL27 | CTAK/Eskine | CCR10 |

T cell differentiation[31]. In contrast, chemokines such as CCL21 or CCL19 that are involved in physiological homing of leukocytes to lymphoid tissues are classified as homeostatic chemokines[33, 35]. The homeostatic group of chemokines modulates lymphocyte and dendritic cell trafficking during immune surveillance[32]. All members of the chemokine family work in concert with selectins and integrins to sort and direct effector leukocyte migration[7, 30].

Chemokines can interact with only one receptor or a single chemokine can bind to multiple receptors[7]. Naturally occurring human chemokines are mostly agonists at leukocyte receptors. Recently it was shown that some chemokines, such as CCL7 or CCL26, could function as chemokine-receptor antagonists[12, 37].

## CHEMOKINE RECEPTORS

Chemokines mediate their biologic actions through a family of more than 20 seven-transmembrane-spanning G protein-coupled receptors[33, 43, 45]. Chemokine receptors are designated according to the class of the chemokine ligands they bind, for example, CR, CCR, CXCR, and CX3CR (Table 1). Chemokine receptors have a distinct chemokine specificity. Furthermore, chemokine receptors show restricted expression on subclasses of leukocytes and non-hematopoietic cells. The ligand specificities of the receptors can substantially overlap within a chemokine class, leading to a high degree of redundancy[7, 35]. In general, the proinflammatory chemokine receptors have more promiscuous ligand-binding specificities, while receptors involved in normal leukocyte trafficking have fewer ligands. In addition, it has been proposed that some chemokine receptors form homo- or heterodimers that may alter their function or sensitivity to chemokine stimulation[7, 35].

## CHEMOKINES REGULATE LEUKOCYTE TRAFFICKING

Chemokines mediate leukocyte-endothelium interaction and transmigration at multiple stages[35, 47]. Injured renal cells produce inflammatory mediators that enhance the expression of adhesion molecules on endothelial cells of capillaries adjacent to the inflammatory lesion. Selectins and vascular addressins mediate the rolling process of leukocytes along the endothelial surface and thereby allow contact of leukocytes with chemokines. These are tethered on endothelial plasma membranes using heparan sulfate proteoglycans following secretion by activated endothelial or subendothelial renal cells. Chemokines bind to their respective receptors on the surface of the rolling leukocyte. This activates leukocyte-expressed $\alpha_2$-integrins, resulting in firm adhesion of the leukocyte to the endothelial surface as a prerequisite for leukocyte transmigration[30, 35, 51]. Other chemokine receptors appear to differentially influence spreading, diapedesis, and subsequent migration into the tissue space[35, 65]. For example, monocytes and T helper (Th)1-like T cells express both chemokine receptors CCR1 and CCR5[64]. 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[64]. 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[40]. Furthermore, chemokines such as CCL5 up-regulate the secretion and activity of matrix metalloproteinases by infiltrating leukocytes[26], thus facilitating leukocyte transmigration through the basement membrane and extracellular matrix[27].

## GENERAL PARADIGMS ABOUT THE ROLE OF CHEMOKINES IN RENAL INFLAMMATION

During the last decade a large amount of data has accumulated on the expression profiles of chemokines and chemokine receptors in several animal models of renal disease as well as in human renal biopsies[6]. Summarizing these data, the following paradigms about the role of chemokines in renal inflammation have been established:

### *All types of renal cells can produce chemokines upon stimulation*

Numerous *in vitro* studies have shown that all types of intrinsic renal cells, including mesangial cells, endothelial cells, podocytes, tubular epithelial cells, and interstitial fibroblasts, can produce proinflammatory chemokines upon stimulation (reviewed by Segerer et al.[47]). In general, proinflammatory stimuli, reactive oxygen species, growth factors, and vasoactive agents such as angiotensin II can stimulate chemokine production of renal cells[47]. Furthermore, immune complexes and complement activation cause mesangial production of chemokines[47]. In proximal tubular cells chemokines can be induced by lipopolysaccharides (LPS)[57], high concentrations of albumin[63, 68], or exposure to both calcium oxalate and calcium phosphate crystals[58]. Chemokines secreted during the early phase of injury may target intrinsic renal cells. The local production of CXCL10 induces proliferation of mesangial cells presumably via CXCR3[42]. CCR7, the receptor for CCL19 and

CCL21, is expressed on mesangial cells and mediates proliferative and antiapoptotic effects[9]. The functional role of CCR1 expression by mesangial cells upon stimulation by CCL5 remains to be elucidated *in vivo*[8]. Besides intrinsic renal cells, infiltrating leukocytes are a major source of local chemokine production in a positive amplification loop[5, 59], as chemokines secreted by infiltrating leukocytes promote additional leukocyte recruitment. In fact, targeting single chemokines or chemokine receptors in animal models of renal disease reduced renal leukocyte recruitment, which is associated with a decrease of local chemokine production[4].

*Chemokine expression localizes to the injured compartment of the kidney*

Several studies have characterized the expression patterns of chemokines in animal models of acute glomerular or tubulointerstitial disease. They demonstrated that various inflammatory chemokines such as CCL2, CCL3, CCL4, and CCL5 are expressed only in the diseased compartment of the kidney (reviewed in Segerer et al.[47]). The spatial expression of chemokines in the kidney correlates with the local accumulation of inflammatory cell infiltrates and renal damage[5, 41, 59]. The Duffy antigen receptor for chemokines (DARC) binds multiple chemokines, including CCL5, but lacks signaling function. In the normal kidney, DARC is expressed at post-capillary venules[48]. During various forms of renal diseases, DARC expression expands to most peritubular vessel endothelium[48]. DARC serves as an endothelial presentation molecule for chemokines involved in signaling of specific exit sites for chemokine-positive cells into the renal interstitium. The relevance of the animal data for human disease has been confirmed by a variety of human biopsy studies[14, 19, 28, 46].

*Chemokines mediate initiation and progression of renal disease*

The course of progressive renal disorder can be divided into 4 phases: initiation, amplification, progression, and the terminal phase[6] (Table 2). In this process; there are roles for chemokines at multiple steps. The contribution of chemokines should be viewed as a part of an integral system together with other cytokines and adhesion molecules. To date, the mechanisms that control resolution or persistence of a leukocytic infiltrate in the kidney have not been elucidated. During acute glomerulonephritis, termination of the trigger injury correlates with a reduction of chemokine expression by intrinsic renal cells and infiltrating leukocytes[5] (Table 2). As further influx of leukocytes does not occur, the number of infiltrating leukocytes declines in parallel with the resolution of glomerulonephritis[5]. It is important to note that termination of the chemokine signal is critical for the resolution of the inflammatory process. On the other hand, 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[1], which recognize components of the infectious organism such as LPS, peptidoglycanes, and unmethylated DNA[1]. We recently showed that injection of CpG-oligonu-

**Table 2.** Stages of renal disease progression

| Phase | Renal function | Pathophysiological mechanisms |
|---|---|---|
| Initiation phase | normal renal function | – initial injury, e.g. immune complexes, toxins, hypoxia<br>– renal cells release chemokines<br>– chemokine fixation on activated endothelium<br>– adhesion of leukocytes |
| Amplification phase | renal dysfunction | – infiltrating cells secrete more chemokines and mediators<br>– glomerular spill–over of chemokines via tubuli and vessels<br>– proliferation of macrophages, fibroblasts, mesangial cells<br>– extracellular matrix secretion<br>– damage of glomerular tuft and podocytes<br>– proteinuria |
| Progression phase | chronic renal failure | – persistent injury, hypoxia, proteinuria, misdirected filtration<br>– continued chemokine expression<br>– loss of capillaries leading to local hypoxia<br>– irreversible structural damage<br>– progressive loss of renal function |
| Terminal phase | end–stage renal failure | – severe structural damage and renal fibrosis<br>– severe hypoxia |

cleotides mimicking bacterial DNA into mice with otherwise self-limiting immune complex glomerulonephritis resulted in a severe exacerbation and progression instead of resolution of the disease process. This was associated with increased chemokine and chemokine receptor expression in the diseased kidneys[2]. Thus, 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 (Table 2).

## IDENTIFYING CCR1 AND CCR2 AS POTENTIAL TARGETS IN KIDNEY DISEASE

The functional roles of various chemokines during renal inflammation were examined by either blocking chemokine activity with neutralizing antibodies, chemokine receptor antagonists, or targeted disruption of genes encoding chemokines and their receptors in various animal models. A detailed survey of these studies has been presented elsewhere[6]. It turned out that within the large family of chemokines and chemokine receptors, two factors appear to be particularly suitable targets for an antagonistic strategy in progressive renal disease. The two factors are the chemokine receptors CCR1 and CCR2.

*Chemokine receptor CCR1*

CCR1 binds several CC chemokines including CCL3, CCL5, CCL7, CCL8, CCL13, CCL14, CCL15, CCL16, and CCL23 in humans[39] (Table 1). CCR1 is expressed at high levels on circulating monocytes and infiltrating tissue macrophages[19, 25, 67]. In view of the role of CCR1 in leukocyte adhesion to activated endothelium as an early event of leukocyte-endothelium interaction, it was suggested that CCR1 may be a non-redundant mediator of leukocyte recruitment to inflamed tissues[64]. Human renal biopsy studies have localized renal CCR1 expression to macrophages in inflammatory cell infiltrates, supporting a potential role for CCR1 in renal inflammation[19]. The first study that used the small-molecule CCR1 antagonist BX471 in kidney disease was reported in 2001 by Horuk et al.[24] In this study BX471 had beneficial effects on renal survival in a model of renal allograft transplantation in rabbit[24]. We have recently studied the effects of BX471 in the model of interstitial fibrosis after unilateral ureteral obstruction (UUO) in mice[4]. UUO kidneys from BX471-treated mice (days 0–10 and days 6–10) revealed a marked reduction of interstitial leukocyte recruitment. Markers of renal fibrosis, such as interstitial fibroblasts, interstitial volume, mRNA, and protein expression for collagen I, were all significantly reduced by BX471-treatment compared with vehicle controls. By contrast treatment was ineffective when the drug was supplied only from days 0–5. Most interestingly, late onset of CCR1 blockade was also effective, identifying CCR1 as a promising target for reducing cellular infiltration and renal fibrosis as major factors in the progression to end-stage renal failure. These data were confirmed by similar studies with CCR1-deficient mice using the UUO model[70]. However, chemokines are also involved in systemic immune responses[67], so that data from the unilateral ureteral obstruction 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[56]. We therefore studied the effects of therapeutic CCR1 blockade in progressive renal injury of lupus-like nephritis in MRLlpr/lpr mice, an autoimmune disease that leads to progressive immune complex glomerulonephritis with tubulointerstitial disease resulting in end-stage renal failure reflecting human lupus nephritis (Anders et al., manuscript submitted). We have recently characterized the expression of chemokines and chemokine receptors during the course of this model and found that, amongst other chemokine receptors, CCR1 and its chemokine ligands are increasingly expressed in kidneys of MRLlpr/lpr mice[41]. When BX471 treatment was initiated late during the course of disease (20–24 weeks of age) BX471 improved blood urea nitrogen levels and reduced the amount of macrophages and lymphocytes in the interstitium. Cell transfer studies with fluorescence-labeled T cells and macrophages that were pretreated with either vehicle or BX471 confirmed that BX471 blocks macrophage and T cell recruitment to the renal interstitium of MRLlpr/lpr mice. 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-β1. BX471 did not affect serum DNA autoantibodies, proteinuria, or markers of glomerular injury in MRLlpr/lpr mice. This represents the first evidence that, in advanced chronic renal injury, blockade of CCR1 can halt disease progression and improve renal function by selective inhibition of interstitial leukocyte recruitment and fibrosis. Despite the multiple roles of chemokines and their receptors in systemic immune responses, CCR1 blockade had no effect on systemic autoimmunity in MRLlpr/pr mice.

Proteinuria has always been considered to be a major prognostic factor for the progression of renal disease.

Albumin induces chemokine expression in renal tubular cells, indicating that proteinuria may serve as a major factor for tubulointerstitial inflammation[50]. We therefore sought to examine the effects of CCR1 blockade with BX471 in a model of focal segmental glomerulosclerosis (FSGS) with nephrotic syndrome and progressive interstitial fibrosis. FSGS was induced in BALB/c mice by two intravenous injections of adriamycin at day 0 and 14. BX471 treatment was started from day 14, when proteinuria had developed. Again, BX471 reduced the amount of interstitial macrophages and T cells and markers of renal fibrosis including interstitial fibroblasts and interstitial volume[60]. In contrast, the extent of glomerular sclerosis and proteinuria was not affected by BX471 treatment. These findings demonstrate that the beneficial effects of therapeutic CCR1 blockade are independent of severe proteinuria.

Together, these data indicate that CCR1 mediates macrophage recruitment into the renal interstitium during renal inflammation. Late onset of treatment with a small-molecule CCR1 antagonist does effectively impair renal leukocyte recruitment and renal fibrosis in various models of progressive renal injury. Human renal biopsy studies of various types of renal disease indicate that human expression of CCR1 reflects what has been found in rodent disease models[19, 28].

*Chemokine receptor CCR2*

Human CCR2 binds CCL2, CCL7, CCL8, CCL13 and CCL16 chemokines[39]. CCL2, an important ligand of CCR2, is expressed by tubular epithelial cells and infiltrating leukocytes in animal models of progressive nephropathies and renal fibrosis[41, 59, 69]. Human renal biopsy studies have confirmed such an expression pattern for CCL2 in various human kidney diseases[6, 20, 21, 46]. Although diabetic and vascular nephropathies are two major causes of progressive renal disease, only a few studies have been published on the role of chemokines in these disorders. An increased expression of CCL2 was reported in human diabetic nephropathy that correlated with the degree of tubulointerstitial damage and macrophage infiltration[10, 44, 61]. In angiotensin II-dependent rat models of hypertensive nephrosclerosis, CCL2 expression was increased and was temporally and spatially related to macrophage infiltration[23]. Based on the similarity of human and rodent CCL2/CCR2 expression, interventional studies performed in rodent models of kidney disease may reflect what can be expected when targeting CCR2 in humans. For example, neutralizing antibodies against CCL2 reduced infiltration of glomerular macrophages in rat anti-Thy-1.1 nephritis[66]. Treatment with anti-CCL2 antibodies reduced proteinuria and monocyte influx in rat nephrotoxic serum nephritis[16, 52] and abrogated crescent formation and leukocyte infiltration in murine nephrotoxic nephritis[29]. A similar reduction of tubulointerstitial damage was obtained with a CCL2 antisense approach in rats with nephrotoxic serum nephritis[38]. Surprisingly, studies with mice with deletion of the gene encoding for this chemokine have yielded partially conflicting results[22, 29, 53, 54]. For example, mice with targeted gene deletion of CCL2 had reduced interstitial leukocyte infiltration and tubular injury but not glomerular injury during the nephrotoxic serum nephritis[53]. In contrast, another group found a marked reduction of crescent formation with a CCL2 blocking antibody in this model[29]. The MRL/MpJ Fas lpr/lpr (MRL/lpr) mouse, which serves as a model of systemic lupus, developed a chronic progressive immune complex nephritis[41]. When CCL2-deficient mice were cross--bred with MRL/lpr mice, lack of CCL2 reduced proteinuria, renal damage, and renal macrophage and T cell recruitment[54]. This suggests that CCL2 plays an important role in the leukocyte infiltration and resulting tissue injury in the lupus model. Human studies in patients with lupus nephritis also showed that urinary CCL2 levels correlated with the extent of renal disease activity and macrophage infiltration[36, 62].

In mice lacking the CCR2 receptor, renal pathology after nephrotoxic serum was worse despite reduced glomerular macrophage infiltration, indicating that lack of CCR2 may influence other immune mechanisms besides the local cell infiltration[11]. After ischemia-reperfusion renal injury the number of interstitial infiltrated macrophages was markedly smaller and the area of tubular necrosis was significantly lower in CCR2-deficient mice than that of wild-type mice[17]. Furuichi et al.[18] have developed a gene construct, 7ND, that encodes for a truncated CCL2 protein and blocks the activation of CCR2 by all of its endogenous ligands, including CCL2, CCL7, CCL8, and CCL13. Systemic or local expression of 7ND after transfection demonstrated beneficial effects in rat protein-overload nephropathy and ischemia-reperfusion injury of the mouse[18, 49]. Improved renal outcome was associated with fewer interstitial CCR2-positive macrophages in both studies. Together, these data indicate that blocking CCR2 effectively impairs renal macrophage recruitment, which reduces renal injury in various disease models. Human renal biopsy studies that examined the spatial expression of CCR2 found similar CCR2 expression to that found in rodent disease[46].

## STRATEGIES TO TARGET NON-REDUNDANT CHEMOKINES OR CHEMOKINE RECEPTORS

To target the chemokine system, several strategies can be applied (Table 3). As mentioned above, for

**Table 3.** Potential strategies to target the chemokine system

| |
|---|
| Blocking leukocyte adhesion to the vascular endothelium and transendothelial migration: |
| CCR antagonists, chemokine analogs, neutralizing antibodies |
| Inhibition of local chemokine production by intrinsic renal cells/infiltrating leukocytes: |
| blocking leukocyte recruitment as above, |
| targeting pattern-recognition receptors that mediate local chemokine expression |
| Selective elimination of chemokine target cells identified by a certain CCR: |
| bispecific antibodies that induce lysis of the target cells by ligating it to cytotoxic T cells, |
| exotoxin-linked CCR-antibodies |

the blockade of the leukocytic cell infiltrate in chronic renal disease, the chemokine receptor CCR1 has been identified as a suitable target. Tokuda et al.[55] have developed a polyclonal antibody with blocking activity against CCR1 that reduced bleomycin--induced pulmonary fibrosis in mice. Others and we have found beneficial effects of CCR1 blockade with a small-molecule antagonist against human and murine CCR1 in various models of renal disease[4, 24]. Studies with other CCR1 antagonists using other immune-mediated disease models (such as collagen--induced arthritis) support the hypothesis that CCR1 blockade may be an effective approach for the therapy of inflammatory disease states that involve tissue damage by local leukocyte infiltration[34, 55]. Shimizu et al.[49] have used a vector construct that produces a truncated CCL2 protein which blocks CCR2 activation. As mentioned before, after transfection of this vector into the femoral muscle of BALB/c mice, ischemia-reperfusion injury of the kidney was dramatically reduced in association with attenuation of renal macrophage recruitment[49]. An additional therapeutic strategy, especially after the leukocyte infiltration has already occurred, could be the targeted elimination of immune cells identified by a specific chemokine receptor. Depletion of CCR5-positive T cells and monocytes is possible by bispecific single-chain antibodies that bind simultaneously to CCR5 on the target cell and CD3 on T cells, thus activating the T cell to eliminate the CCR5-positive leukocytes[13]. Another possibility to eliminate CCR5-positive cells is represented by a fusion protein consisting of the chemokine CCL5 and a truncated version of *Pseudomonas* exotoxin A, which specifically destroys the target cell after binding to their CCL5 receptors, such as CCR5[13]. Elimination of leukocyte subsets identified by a particular chemokine receptor within the inflamed tissue may represent a novel therapeutic strategy of selective immunosuppression. This approach would avoid systemic side effects secondary to leukopenia or systemic immunosuppression.

However, chemokine antagonists may induce adverse reactions. For example, we have recently shown that CCL5 peptide antagonist can aggravate glomerular damage and proteinuria in mice with immune complex glomerulonephritis despite a reduction of glomerular leukocyte infiltration[3]. Exacerbation of renal disease by targeting of single chemokine receptors has also been found in knockout mice for the CCR1 and CCR2[11, 56]. Although effects that occur in chemokine receptor knockout mice may be different from those occurring with short-term use of receptor antagonists, the present data indicate that therapeutic targeting of chemokine receptors, under certain conditions, may exacerbate underlying disease. Beneficial effects of chemokine blockade can be explained best by the prevention of leukocyte infiltration, but their effects on the phenotype, survival or immigration of infiltrated cells, as well as their effects on dendritic cells, memory T cells, B cells, and antibody production must be considered. As chemokine receptors are involved in many other physiological processes, potential side effects of receptor blockade must be carefully evaluated before approaching treatment studies in human disease.

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