

Chemokines and chemokine receptors in renal pathology

Stephan Segerer^a and Charles E. Alpers^b

Purpose of review

Chemokines are members of the largest group of chemotactic cytokines, and were the first shown to be able to engage specific subpopulations of inflammatory cells. Accordingly, our expanding knowledge in chemokine biology has enlarged our understanding of inflammatory cell interactions, lymphopoiesis, specificity of cell recruitment, and a variety of human diseases. This review covers recent developments on chemokines in renal diseases.

Recent findings

Intrinsic renal cells are capable of chemokine expression *in vitro* and *in vivo*, and the involved induction pathways are becoming increasingly defined. Differential chemokine expression during the time course of disease, followed by an infiltration of cells expressing the corresponding receptors has been described in animal models. Therapeutic efficacy of chemokine blockade has been demonstrated in a variety of disease models, including progressive interstitial fibrosis. Chemokine receptors are differentially expressed and localized to specific parenchymal compartments in human renal diseases, as revealed by studies of renal biopsies, and some functional roles of specific chemokine/receptor interactions can be deduced through the correlation of patterns of expression, genetic variations and disease courses.

Summary

Chemokines play an important role in renal inflammation. Although the treatment of patients with renal diseases using chemokine receptor blocking agents has not yet reached clinical practice, a recent body of data indicates that human renal disease might be amenable to such approaches.

Keywords

chemokines, chemokine receptor, glomerulonephritis, interstitial fibrosis, transplant rejection

Abbreviations

DARC	Duffy antigen/receptor for chemokines
IL-8	interleukin-8
IP-10	γ -interferon-inducible protein-10
I-TAC	interferon-inducible T cell- α chemoattractant
MCP	monocyte chemoattractant protein
Mig	monokine induced by γ -interferon
MIP	macrophage inflammatory protein
PTC	peritubular capillary
RANTES	regulated upon activation, normal T cell expressed and secreted
SDF-1	stromal cell-derived factor-1
SLC	secondary lymphoid tissue chemokine
TLR	toll-like receptor

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Introduction

The chemokines constitute the largest family of small chemotactic cytokines, identified by their ability to attract specific subsets of leukocytes to sites of inflammation [1]. This family has grown to almost 50 members, so far, with 18 corresponding receptors [1,2]. According to variations in a conserved four-cysteine motif, present close to the N-terminus, these chemokines can be segregated into four groups: (1) two cysteines have adjacent locations (CC); (2) two cysteines are separated by a single amino acid (CXC), (3) two cysteines are separated by three amino acids (CX3C), or (4) the C chemokines XCL1/lymphotactin- α and XCL2/lymphotactin- β , which are missing two of the four cysteines. The original and somewhat unwieldy list of chemokine names has recently been replaced by a systematic nomenclature for ligands, and their corresponding receptors (Fig. 1). In this review we use both names together, as the older nomenclature is still widely employed [1]. Chemokine receptors bind and respond to chemokines of a particular subfamily and are correspondingly designated (Fig. 1).

Chemokines can bind to acidic macromolecules like glycosaminoglycans, via basic amino acids in the core and the C terminus. This enables chemokines to be presented on the surface of cells, with full chemotactic activity [3•]. Like other cytokines, chemokines are secreted, and function locally in either paracrine or autocrine fashion [4]. In addition to the subfamilies defined by protein structure above, chemokines can also be broadly divided into two functional chemokine subsets: (1) chemokines that are constitutively expressed and are involved in lymphoid homeostasis, and (2) inducible chemokines, which are upregulated during inflammatory responses [5••]. Most of the literature on chemokine activity in the kidney is focused on inducible

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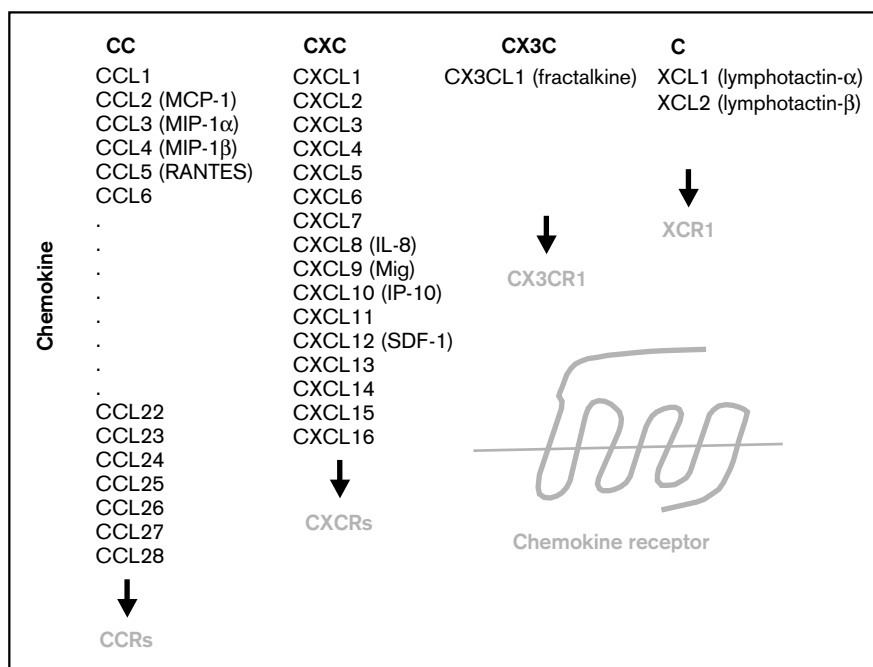
^aNephrological Center, Medical Policlinic, University of Munich, Munich, Germany and
^bDepartment of Pathology, University of Washington, Seattle, Washington, USA

Correspondence to Charles E. Alpers, University of Washington, Department of Pathology, Medical Center, Box 356100, 1959 NE Pacific Street, Seattle, WA 98195, USA
Tel: +1 206 598 6409; fax: +1 206 598 4928; e-mail: calp@u.washington.edu

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Figure 1. Current nomenclature of the chemokines and their corresponding receptors

Chemokines function via subgroup specific, seven-transmembrane spanning receptors. L, ligand; MCP, monocyte chemoattractant protein; MIP, monocyte inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted; IL, interleukin; Mig, monokine induced by γ -interferon; IP-10, γ -interferon-inducible protein-10; R, receptor; SDF-1 stromal cell-derived factor-1.



chemokines and their corresponding receptors, but a role for homeostatic chemokines and their receptors, is beginning to evolve [6•].

The data on chemokines in renal diseases have been reviewed extensively [4,7,8]. Here we consider some recent aspects in general chemokine biology, followed by a review of recent studies on the role of chemokines in renal cells *in vitro*, in animal models, in human renal biopsies, in renal development, and the role of chemokine polymorphisms in human renal diseases.

Recent developments in chemokine biology

It is now well established that a cascade of chemokine/chemokine receptor interactions is involved during thymocyte development. CCR7 and its ligands play pivotal roles in the recirculation of naive T cells via high endothelial venules, and the localization of these cells to specialized microenvironments in lymph nodes [5•,9,10]. Furthermore, CCR7 is involved in the migration of activated dendritic cells from sites of injury, via lymphatic vessels, towards T cell areas of secondary lymphoid tissues [5•]. Through this fine tuned trafficking of dendritic cells and T cells, chemokines are a link between innate and adaptive immunity [11•].

Chemokine activity in innate immunity is further supported by studies of pattern-recognition receptors such as toll-like receptors (TLRs). These receptors recognize conserved antigenic motifs, commonly found

in bacteria and prokaryotic organisms, and when engaged lead to the induction of chemokines [11•,12•]. TLRs might be important for host response to infections, and there is new evidence to suggest that they might also be responsible for flares of renal diseases during infections not directly involving the kidney (see below).

Chemokine induction and regulation in renal cells *in vitro*

Proteinuria is a well established progression factor in renal disease. The expression of chemokines by tubular epithelium as a consequence of proteinuria has been suggested as one key to the interstitial inflammatory infiltrates found in progressive renal diseases. Tubular epithelial cells can be a rich source of inflammatory chemokines including CCL5/RANTES, CCL2/MCP-1, CCL3/MIP-1 α , CX3CL1/fractalkine and CXCL8/IL-8 [4,13,14]. To further define the involved pathways, tubular epithelial cells were exposed to either human albumin or immunoglobulin G, causing a dose-dependent release of hydrogen peroxide which then mediated activation of nuclear factor- κ B [15•]. Protein kinase C inhibitors prevented hydrogen peroxide production as well as induction of CCL2/ monocyte chemoattractant protein (MCP)-1 [15•].

Production of functional chemokines by cultured tubular epithelial cells has been demonstrated by cell migration assays [16]. Migration of activated peripheral blood mononuclear cells towards supernatants obtained from

cytokine stimulated tubular epithelial cells has been demonstrated, and enzyme-linked immunosorbent assays and blocking have identified CCL5/RANTES (regulated upon activation, normal T-cell expressed and secreted), CXCL10/ γ -interferon-inducible protein-10 (IP-10), and CX3CL1/fractalkine as responsible chemokines [16]. These in-vitro data are concordant with patterns the chemokine receptor expression identified in the interstitium in human diseases.

Tubular epithelial cells are also targets for chemokines, as they respond to CCL2/MCP-1 exposure with induction of interleukin-6 and intercellular adhesion molecule-1 [17•]. The induction of these molecules involved Gi-proteins, protein kinase C and nuclear factor- κ B signalling. The involved chemokine receptors remain unknown. These data suggest that an autocrine loop might perpetuate the deleterious effect of proteinuria, and in aggregate suggest that blockade of protein kinase C could interfere at two different steps (i.e. hydrogen peroxide release by tubular epithelial cells after protein stimulation, as well as induction of a pro-inflammatory phenotype after CCL2/MCP-1 exposure) of the injury process [18].

TLRs might be important for the secretion of chemokines after exposure of renal cells to bacterial components. Primary murine renal tubular epithelial cells express TLRs 1–4, and 6 but not TLR5 and 9 [12••]. Induction of CCL2/MCP-1 and CCL5/RANTES has been demonstrated after exposure of these cells to synthetic lipid A and lipoprotein, implying a function for TLR4 and TLR2 [12••].

An additional stimulus for the expression of CCL2/MCP-2 by tubular epithelial cells can be oxalate ions and crystals of calcium oxalate, encountered in urolithiasis and multiple types of advanced kidney diseases [19].

Expression of chemokine receptors by intrinsic renal cells *in vitro*

CCR4, CCR8, CCR9, CCR10, CXCR1, CXCR3, CXCR4 and CXCR5 messenger RNAs were detected in cultured human podocytes by reverse transcriptase–polymerase chain reaction [20••]. Surprisingly, in glomerular RNA isolated from normal kidneys, the authors demonstrated the expression of CCRs 1–10, as well as CXCR1 to CXCR5 using the same technique. Functionality of the expressed chemokine receptors *in vitro* was demonstrated for the CCRs and CXCRs 3–5 by an increase of free cytosolic calcium after exposure to the corresponding ligands. CXCR 3–5 protein was confirmed by immunofluorescence on cultured primary and immortalized podocytes. Exposure to the corresponding ligands led to an induction of superoxide anion production. Finally, the authors used immunofluorescence of

normal renal cortex and cortex from two patients with membranous nephropathy and found expression of CXCR1, CXCR3 and CXCR5 in podocytes from patients with membranous nephropathy. Further studies are clearly needed to confirm these important results. The finding that all chemokine receptors are expressed in normal glomeruli conflicts with previous studies using immunohistochemistry or in-situ hybridization that failed to detect CCR5, CCR2, CXCR4 or CXCR3 in these cells [21–23].

Induction of CCR2 (in addition to CCR1), as well as functional CXCR2 has been demonstrated on primary cultured mesangial cells [24]. Previously, the expression of CCR1, but not CCRs 2–5, was described in immortalized mesangial cells and in primary mesangial cells [25]. Using different tissues and approaches, our studies on the expression of CCR2 using in-situ hybridization did not demonstrate CCR2 expression in mesangial cell and the significance of the data on CCR2 and CXCR2 awaits further studies [26,27]. The first receptor for constitutively expressed chemokines, CCR7, was recently described on mesangial cells and a functional role in glomerular homeostasis was suggested [6••]. Interestingly the corresponding ligand CCL21/secondary lymphoid tissue chemokine (SLC) was found to be constitutively expressed by podocytes. Human mesangial cells demonstrated migration, increased proliferation, a decrease in apoptotic cell death, and a decreased time of wound closure in response to CCL21/SLC [6••].

Animal models and therapeutic impact of chemokine blocking agents

In a prolonged anti-Thy-1 rat model of mesangiolysis and repair, expression of CX3CL1/fractalkine by glomerular endothelium was found early in the disease course, and was localized to mesangial cells at later time points [28]. Corresponding glomerular infiltration by CX3CR1 expressing macrophages was demonstrated. Candesartan, an angiotensin II type I receptor antagonist, diminished both the expression of CX3CL1/fractalkine, and the infiltration by CX3CR1 positive cells early in the disease course. This study is noteworthy for the demonstration that there may be significant effects on the chemokine system as an unintended effect of drugs widely used to treat renal disease through blockade of the renin–angiotensin system.

After temporary ischemia the induction of several mouse chemokines, including KC (a murine homologue of human Gro proteins), was previously described, and the blockade of KC and monocyte inflammatory protein-2 has now been studied in further detail [4,29•]. Ischemia and reperfusion induced a rapid induction of KC, and

treatment with blocking antibodies decreased early neutrophil influx, prevented deterioration of renal function, and decreased mortality [29•]. The blocking antisera were given just before reperfusion, an approach that might be applicable to ameliorating early allograft injury in the setting of renal transplantation.

Blockade of CCL5/RANTES activity with the competitive antagonist Met-RANTES significantly reduced vascular and tubular injury in a rat model of acute renal allograft rejection [30]. To extend these studies, rats treated with cyclosporin A received either low doses or high doses of Met-RANTES, or vehicle after transplantation [31••]. The Met-RANTES treated groups demonstrated reduced inflammatory cell infiltration, and reduced cytokine expression after 2 weeks. Furthermore, Met-RANTES treated animals demonstrated reduced proteinuria, amelioration of glomerulosclerosis, and interstitial injury, reduced transplant vasculopathy, and decreased cell infiltration at 28 weeks after transplantation. These studies indicate the possible benefits of chemokine antagonism to prevent both acute rejection and the development of chronic allograft nephropathy. Whether treatment after chronic allograft nephropathy is established prevents further progression has not been studied. A detailed review of the role of chemokines in allograft rejection is beyond the scope of this paper, but excellent reviews in this field were recently published [8,32••].

Important evidence on the role of TLRs in renal diseases comes from a study of immune complex glomerulonephritis induced by apoferritin in mice [33••]. The injection of CpG-oligonucleotides (a motif common to prokaryotic organisms and which binds to TLR9) resulted in a worsening disease, including increased glomerular macrophage infiltration, and increased expression of chemokines (e.g. CCL2/MCP-1 and CCL5/RANTES). Furthermore, expression of the corresponding receptor for CpG oligonucleotides (i.e. TLR9) was demonstrated on monocytes/macrophages, but not in mesangial cells. A mechanism by which infection can lead to flares or aggravation of coexisting renal diseases is provided by these data.

Chemokine and chemokine receptor expression in human renal biopsies

Differential expression of chemokine receptors by leukocytes infiltrating the kidney in various diseases was demonstrated in a number of studies. The chemokine receptor CCR5 is mainly expressed by infiltrating T cells in the interstitium, whereas the distribution of CCR2-expressing cells corresponds to the distribution of monocytes/macrophages [21,27]. Recently the distribution of fractalkine and the corresponding receptor, CX3CR1, were described

[13,34•,35•]. CX3CL1/fractalkine was found to be upregulated in the predominant compartment during renal injury: in glomeruli during vasculitic glomerulonephritis and the interstitium during acute allograft rejection [13,35•]. CX3CL1/fractalkine messenger RNA was expressed by tubular epithelial cells during allograft rejection and most likely by endothelial cells of peritubular capillaries and glomeruli. Cellular infiltrates of macrophages and T cells co-localized with sites of CX3CL1/fractalkine expression [35•]. CX3CR1 expression was found on the majority of infiltrating mononuclear leukocytes in different renal compartments in various glomerular diseases and renal allograft rejection. In glomerular diseases with prominent macrophage infiltration of the glomeruli, the distribution of monocytes/macrophages matched the distribution of CX3CR1. Additionally, in interstitial infiltrates the distribution of CX3CR1 corresponded to the distribution of both T cells and monocytes/macrophages. The distribution pattern of CX3CR1 expressing cells was consistent with the distribution described for the corresponding ligand described above [34•]. These data argue for a hypothetical model in which CX3CL1/fractalkine acts as an adhesion molecule early in the extravasation cascade, whereas the ligands for CCR5 and CCR2 subsequently might guide inflammatory cells to more specific renal compartments.

The expression of CXCR3, a receptor for the chemokines CXCL10/IP-10, CXCL9/monokine induced by γ -interferon (Mig), and CXCL11/interferon-inducible T cell- α chemoattractant (I-TAC), was previously described on mesangial cells in renal biopsies of patients with mesangioproliferative glomerulonephritis, results which still await confirmation using different antibodies or techniques [23]. Strong expression of CXCL10/IP-10 and CXCL9/Mig was described in glomeruli and infiltrating cells of kidneys with membranoproliferative glomerulonephritis, crescentic glomerulonephritis, and immunoglobulin A nephropathy, but not membranous nephropathy and normal kidneys [36]. Double labelling suggested that mesangial cells were the main source of both chemokines; in addition CXCL9/Mig immunoreactivity and to a lesser extent CXCL10/IP-10 were found on podocytes. Infiltrating monocytes/macrophages also expressed these two chemokines.

The Duffy antigen/receptor for chemokines (DARC) represents the Duffy blood group on red blood cells and is able to bind a multiplicity of chemokines, lacking the subgroup specificity of the identified chemokine receptors. DARC is expressed on specialized endothelial cells, which are present at the site of cell homing on high endothelial venules, and at a major site of inflammatory cell recruitment into the kidney, the peritubular capillaries (PTCs) [4,37]. It was previously demonstrated

that the number of DARC expressing PTCs is increased in human allografts with acute interstitial and acute vascular rejection [38]. The deposition of the complement split product C4d in combination with an accumulation of inflammatory cells in PTCs was considered to be a sign of human humoral allograft rejection. As DARC might be involved in the recruitment of inflammatory cells into PTCs during humoral rejection, the expression of DARC was compared, in allograft biopsies without rejection, with humoral rejection, with cellular rejection, and with a mixed pattern of both humoral and cellular rejection [39]. Both forms of allograft rejection led to a similar increase in the number of DARC positive PTCs. The highest numbers of DARC positive vessels were found in patients with C4d deposits and Banff grade 1 rejection. Therefore, DARC is upregulated in all forms of acute human allograft rejection with a potentially different regulation in humoral and cellular rejection.

Impact of chemokine gene polymorphism on the outcome on human renal diseases

The first study on the role of the 32 base pair deletion of CCR5, called CCR5-Δ32, described a cohort of 576 recipients of a renal transplant. Twenty-one patients were homozygous for CCR5-Δ32 and 555 were heterozygous or had the wild type allele. Only one patient in the CCR5-Δ32 homozygous group lost the allograft during follow up, as compared with 78 in the control group, and renal allograft survival was significantly better in the CCR5-Δ32 homozygous group [40]. Additional chemokine receptor gene variants, namely CCR5-59029-A/G, CCR2-V64I, CX3CR1-V249I and CX3CR1-T280M were evaluated in a cohort of 163 renal allograft recipients [41•]. A reduction in the risk for acute allograft rejection was found in recipients homozygous for the CCR5-59029-A allele and the recipients with the CCR2-64I allele, but not with variations of the CX3CR1 allele [41•]. No impact of the CCR2-64I polymorphism was found on allograft survival in a different cohort, seemingly in contrast to the above mentioned results which demonstrated a reduction in the number of acute rejection episodes [41•,42•]. Homozygotes with the G/G allele of MCP-1-2518 had a reduction of the mean graft survival as compared with the heterozygotes (A/G) and patients with the wild-type allele (A/A) [42•]. In aggregate these studies help to define the relative importance of CCR5, CCR2 and the corresponding ligands in mediating human allograft rejection, and also help to identify recipients with increased risk of allograft rejection when these pathways are modified by genetic variations.

A single nucleotide polymorphism of the CXCL8/interleukin-8 gene was more frequent in African-Americans with severe lupus nephritis than in patients with

less severe nephritis, and controls [43], but the pathophysiologic basis of this finding remains obscure.

The role of chemokines during renal development

A comprehensive study on the expression of CXCL1/fractalkine, CXCL12/stromal cell-derived factor-1 SDF-1, CXCL10/IP-10 and the corresponding receptors CX3CR1, CXCR4 and CXCR3, described a differential pattern of expression during human renal development [44•]. Experimental proof for a functional role of chemokines during renal development or renal malformations is still missing as mice deficient in some of these molecules, such as CX3CL1 and CX3CR1, demonstrated normal renal development.

Role of chemokines in HIV associated nephropathy

HIV associated nephropathy is the most common form of renal disease among HIV-1 seropositive patients of African-American descent [45]. It is characterized by a collapsing form of focal and segmental glomerulosclerosis, commonly with microcystic dilation of renal tubules, interstitial inflammatory infiltrates and fibrosis [45]. Several recent studies demonstrated that HIV-1 can be detected in renal biopsies from affected patients in tubular epithelial cells, parietal and visceral epithelial cells [46,47]. These studies demonstrate that the kidney might be an important reservoir for HIV. It is still unclear how renal cells become infected by HIV, as the main co-receptors for HIV are not expressed at detectable levels (e.g. through immunohistochemistry or in-situ hybridization) in intrinsic renal cells [48,49]. Defining how HIV infects parenchymal cells in the kidney might be important for new therapeutic approaches to HIV associated renal diseases.

Future perspectives

Studies on chemokines utilizing in-vitro approaches, animal models, and human renal biopsies have suggested pathways of recruitment of leukocytes into the kidney, but we are still only beginning to understand the patterns of chemokine receptors on inflammatory cells, and their role in renal diseases. We anticipate that the combination of real-time reverse transcriptase-polymerase chain reaction, laser capture microscopy, and immunohistology will help combine sensitive, quantitative messenger RNA expression and morphological localization in renal tissue and enhance our understanding [50•].

As a role for chemokines mediating apoptosis and proliferation has become apparent, chemokine expression in different renal compartments (e.g. in glomerular diseases as well as in the tubular injury of chronic kidney diseases) as these processes are initiated needs to be addressed.

There is no doubt that chemokines play an important role in dendritic cell biology, but the role of dendritic cells during renal diseases, and especially renal allograft rejection has yet to be addressed adequately.

Conclusion

Chemokines are members of the largest group of chemoattractants that are specific for subsets of inflammatory cells. Functional roles and the therapeutic impact of chemokine blocking agents have been described in a variety of animal models and in human allograft rejection. In the future, altered expression of chemokines could be exploited both for diagnostic purposes as well as for therapeutic interventions. The studies to date indicate great promise for this approach, but translation into clinical applications remains to be achieved.

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