Renal inflammation underlies many chronic kidney diseases and the infiltration of leukocytes mediates much of the nephritogenic inflammatory response. As several recent reviews have dealt with the basic biology, mouse models, and blockade studies of chemokines in renal diseases,1–11 we focus here on recent developments in pathophysiology of that chemokine effect.

CHEMOKINES AND CHEMOKINE RECEPTORS

Chemokines are a group of chemotactic cytokines (approximately 8 to 17 kD) with the ability to bind G-protein–coupled receptors and act as potent attractants for leukocytes in acute and chronic inflammation. There are four subfamilies of chemokines, including CCL, CXCL, CX3CL, and CI (Figure 1).6,7 At present, 19 receptors are known (Figure 1).6,7 The large CC chemokine family has the first two cysteine residues adjacent to each other, whereas the CXC family has a single amino acid residue in between the first two cysteines. Fractalkine (CX3CL1) is the only member of the CX3C chemokine family where three amino acid residues separate the first two cysteines. Finally, two related chemokines absent the two cysteine residues that bind the XCR1 receptor belong to the XC family. As shown in Figure 1, many chemokines bind multiple receptors and most receptors bind multiple chemokines. However, CC chemokine receptors exclusively bind CC chemokines and CXC receptors bind only CXC chemokines.

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Chemokines and their corresponding receptors are expressed in different cell types (Figure 2).7,12 Generally speaking, monocytes are mostly attracted by CCL chemokines acting through CCR1, CCR2, and CCR5 receptors, whereas neutrophils are the target of CXCL chemokines through CXCR1 and CXCR2 receptors (Figure 2). Inflammatory cells such as T-helper1 (Th1) cells and natural killer cells are attracted by chemokines binding to CXCR3, CXCR6, CCR5, and CX3CR1 to induce local type 1 cytokine (IL-2, INF-γ)–mediated inflammatory responses, whereas Th2 cells and eosinophils are recruited by ligands for CCR3, CCR4, and CCR8 during a type 2 cytokine (IL-4, IL-5, and IL-13)–mediated inflammatory responses. CCR6 and CXCR3 receptors are detected on Th17 cells and a spectrum of chemokines receptors are expressed in regulatory T cells (Tregs), such as CCR4–8, CXCR3, and CXCR6. In kidneys, endothelial cells, podocytes, mesangial cells (MCs), tubular epithelial cells, and interstitial fibroblasts can also produce inflammatory chemokines upon stimulation.3,9

REGULATION OF CHEMOKINE EXPRESSION

On the basis of their regulation and production, chemokines can be classified broadly as homeostatic/lymphoid or inflammatory chemokines. The former is responsible for leukocyte homing and

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lymphocyte recirculation under normal conditions, whereas the inflammatory chemokines contribute to progression of inflammatory diseases.\(^7\) In the normal kidney, production of inflammatory chemokines is low, but is significantly increased under pathophysiological circumstances such as ischemia, toxin exposure, or acute inflammation.\(^3\) Proinflammatory cytokines, such as TNF-\(\alpha\) and IL-1\(\beta\), and reactive oxygen species are major mediators responsible for chemokine expression\(^3,7\) through the NF-\(\kappa\)B pathway,\(^13\) including connective tissue growth factor-stimulated NF-\(\kappa\)B.\(^14\) Inflammatory chemokines are also induced by other mediators, including cyclic adenosine monophosphate, growth factors such as PDGF, basic fibroblast growth factors, pathogen-associated molecules such as lipopolysaccharides, Ig aggregates, LDL, IFN-\(\gamma\), and vasoactive substances like angiotensin II, or under diabetic conditions.\(^7,15\) Activation of TGF\(\beta/Smad2,3\) signaling during renal inflammation also produces a chemotactic effect on macrophages by inducing monocyte chemoattractant protein-1 (MCP-1)/CCL2 expression.\(^16,17\)

Furthermore, urinary levels of TWEAK, a cytokine of the TNF superfamily, significantly increase and correlate with the activity of renal disease in patients with active lupus nephritis.\(^18\) Exposure to TWEAK also induces MCP-1/CCL2, RANTES/CCL5, MIP-1\(\alpha/CXCL2,\) intraperitoneally-10/CXCL10, and SLC/CCL21 in MCs or tubular epithelial cells through a noncanonical NF-\(\kappa\)B pathway, thereby promoting leukocyte recruitment to the kidney during injury.\(^19,18,20\)

The IL-17/IL-23 signaling pathway through Th17 cells also regulates the production of Th1-attracting chemokines and inflammatory cell infiltration. In mouse MCs, IL-17 enhances the production of MCP-1/CCL2, MIP-1\(\alpha/CXCL2,\) LARC/CCL20, and MCP-2/CXCL2 through the MAPK pathways (p38 MAPK and ERK1/2) to recruit T cells and monocytes.\(^21-23\) Mice infused with Th17 cells also develop albuminuria, which is associated with higher levels of renal GRO-\(\alpha/CXCL1\) and neutrophils in glomeruli.\(^24\)

**ROLE OF CHEMOKINES IN ACUTE KIDNEY INJURY**

Rapid accumulation of neutrophils and monocyte/macrophages in injured kidney is an essential feature of the innate immune response induced by ischemia-reperfusion injury (IRI).\(^25\) Several chemokine families...
show a strong relationship to acute kidney injury (AKI), including the CXCL subfamily—IL-8/CXCL8, Gro-α/CXCL1, and MIP-2/Gro-β/CXCL2—that act primarily on neutrophils (Figure 2), the CCL subfamily (MCP-1/CCL2), the CX3CL subfamily (fractalkine/CX3CL1) that have specific effects on monocytes and monocyte-derived lineages, and RANTES/CCL5 that operate more broadly to attract cells monocytes and lymphocytes (Figure 2).

**Role of the CXCL Family in Innate Immune Responses in AKI**

After IRI, an increase in the expression of IL-8/CXCL8 and Gro-α/CXCL1 associates with marked neutrophil infiltration, suggesting that CXCL chemokines play a significant role in neutrophil recruitment. The ability of IL-8/CXCL8 to recruit neutrophils by upregulating tubular ICAM-1, by engaging their cognate receptor, CXCR-1, to activate the p38 MAPK signaling pathway reveals an essential role of the CXCL family in the process of neutrophil recruitment.

The functional role of CXCL family in AKI is clearly demonstrated by finding that treatment with a CXCR2 inhibitor or neutralizing antibodies to Gro-α/CXCL1 or MIP-2/Gro-β/CXCL2 during renal IRI blocks interstitial neutrophil infiltration, reduces renal damage, and improves survival. Deficiency of IL-23, IL-17A, or IL-17 receptor, or the addition of neutralizing antibodies to CXCR2 in mice, attenuates neutrophil infiltration in IRI. During the innate immune response in IRI, neutrophils produce a large amount of IL-17A, which in turn induces proinflammatory cytokines and chemokine (CXCL1/2) to promote kidney inflammation. It is known that IL-17 is able to strongly repress TNF-α-stimulated expression of intraperitoneally-10/CXCL10, 1-TAC/CXCL11, and RANTES/CCL5, but it also acts synergistically with TNF-α to induce IL-8/CXCL8, Gro-α/CXCL1, and MIP-3a/CCL20. Thus, in addition to proinflammatory cytokines, IL-17 and IL-23 signal pathways are new modulators of chemokine-mediated neutrophil infiltration during the innate immune response to IRI, and targeting this pathway may be a new approach for its treatment.

Recent studies also reveal a role for CXCR3 in IRI in mice. CXCR3 is mainly expressed on activated Th1 cells to mediate Th1 recruitment. CXCR3 ligands (intraperitoneally-10/CXCL10 and Mig/CXCL9) are upregulated in postischemic tissue and CXCR3-deficient kidneys with IRI decrease their infiltration of IFN-γ-producing CD4+ T cells as well as the severity of acute tubular necrosis. This protective effect is abrogated by adoptive transfer of wild type CD3+ cells into CXCR3 null mice, demonstrating a critical role for CXCR3 in Th1 cell recruitment to the ischemic kidney. Furthermore, administration of IL-13, a potent Th2 cytokine, also exhibits an inhibitory effect on interstitial infiltration by neutrophils and macrophages, which associates with reduced expression of MIP-2/Gro-β/CXCL2, IL-8/CXCL8, and MCP-1/CCL2, supporting the involvement of CXCL chemokines in the T cell infiltration of AKI.

**Role of CCL and CX3CL Families in AKI**

Presently, it is known that macrophages in the inflamed kidney are either recruited from the circulation or derived from the proliferation of resident monocytes. MCP-1/CCL2, RANTES/CCL5, MIP-1α/CCL3, and MIP-1β/CCL4 are the most commonly described chemokines used in the recruitment of monocyte/macrophages during renal inflammation. The corresponding receptors mediate firm adhesion (CCR1), shape change (CCR2 and CCR5), spreading (CCR2 and CCR5), and transmigration.

A critical role for chemokine signaling in macrophage infiltration is demonstrated by a number of functional blocking experiments. For example, abrogation of CCR1 or CCR2 signaling in a mouse model of AKI results in reduction of interstitial macrophage infiltrates. Delivery of a truncated MIP-1/CCL2 protein into mice with IRI also blocks activation of CCR2, thereby inhibiting macrophage infiltration. Interestingly, injured kidneys from mice lacking CCR1 also reduce expression of CCR1 ligands (MIP-1α/CCL3 and RANTES/CCL5), suggesting the existence of a positive feedback loop for chemokine production.

Several recent reports highlight the protective role of Tregs during IRI. However, the source of the renal Tregs in response to IRI is uncertain. As renal expression of MCP-1/CCL2 is greatly elevated after IRI and CCR2 has been shown to guide Tregs toward arthritic joints, CCL chemokines may function to regulate Treg cell accumulation in acute kidney injury. dendritic cells (DCs) are also an important link between innate and adaptive immunity and their role in renal injury is well known. After IRI, renal DCs produce both the proinflammatory cytokines (TNF-α, IL-6, and CCL) and chemokines (MCP-1/CCL2 and RANTES/CCL5). Thus, secretion of these CCL chemokines from DCs may attract monocyte/macrophase to the site of inflammation.

Increased fractalkine/CX3CL1 expression in injured endothelial cells and blood vessels is also observed in AKI. CX3CR1 is predominantly expressed on macrophages and blockade of CX3CR1 by neutralizing antibody or gene inactivation prevents the kidney from ischemic injury by inhibiting macrophage infiltration, suggesting that fractalkine/CX3CL1 is a potent chemoattractant molecule for macrophages carrying CX3CR1. CCR2 and CX3CR1 are both essential for the acute release of macrophages from the bone marrow for infiltration into injured kidney. Although ablation of CCR2 and CX3CR1 diminishes macrophage infiltration, MCP-1/CCL2 deficiency exerts no effect on renal macrophage infiltration, suggesting the presence of ligand redundancy. Thus, targeting the early macrophage trafficking by CCR2 and CX3CR1 may have therapeutic potential for AKI.

**ROLE OF CHEMOKINES IN CHRONIC KIDNEY DISEASES**

Accumulating data from clinical studies and animal models support the notion that chemokines and their cognate receptors play a critical role in the recruitment of T cells, macrophages, and dendritic cells during the development of chronic renal injury (Figure 2). Although levels of urinary IL-8/CXCL8 ex-
creatinuria are increased in patients in the acute phase of various forms of glomerulonephritis, a large number of CXCR1-positive neutrophils are also found in both glomeruli and the tubulointerstitial tissue of the patients with membranous proliferative glomerulonephritis, lupus nephritis, and crescentic glomerulonephritis. The functional role of CXCL family in inflammatory kidney disease is clearly demonstrated by the finding that administration of an anti-IL-8/CXCL8 antibody to rabbits with an immune complex nephritis reduces proteinuria and neutrophil recruitment.

Role of CCL and CX3CL Chemokines in Chronic Kidney Disease

Increasing evidence suggests a switch of expression of CXCL chemokines (IL-8/CXCL8) to CCL chemokines (MCP-1/CCL2, RANTES/CCL5) in the transition from acute to chronic inflammation (Figure 2). As in AKI, urinary IL-8/CXCL8 levels are increased early, but elevated urinary MCP-1 levels associate with progressive renal injury. In addition, enhanced expression ofglomerular MCP-1/CCL2 and ligands of CCR5 (including RANTES/CCL5, MIP-1α/CCL3, and MIP-1β/CCL4) are detected in patients with various forms of glomerulonephritis.

Increased levels of MCP-1/CCL2 are also associated with progressive tubulointerstitial disease as tubular epithelial cells are rich sources of CCL chemokines, including MCP-1/CCL2, RANTES/CCL5, and MIP-1α/CCL3. In unilateral ureteral obstruction (UUO) producing fibrosis, upregulation of tubulointerstitial MCP-1/CCL2 correlates with degree of macrophage infiltration, which is associated with activation of the TGFβ/Smad3 signaling pathway. TGFβ signals through its downstream mediator, Smad3, to induce MCP-1 expression. Thus, mice deficient in Smad3, an inhibitor of TGFβ/Smad3 signaling, result in enhanced TGFβ/Smad3 signaling through which MCP-1/CCL2-dependent macrophage infiltration and tubulointerstitial fibrosis develop in the UUO kidney. In contrast, inhibition or loss of TGFβ/Smad3 signaling blocks MCP-1 expression and macrophage accumulation in anti-GM1 nephritis and UUO. Thus, upregulation of MCP-1/CCL2 and the development of macrophage infiltration and tubulointerstitial fibrosis correlate with chronic kidney disease.

A critical role for CCL chemokines in progressive renal injury is suggested by functional blocking studies, including treatment with neutralizing antibodies to CCL chemokines (MCP-1/CCL2) or their receptors (CCR2), chemically modified chemokines (RANTES/CCL5), truncated chemokines (MCP-1/CCL2), or small-molecule receptor antagonists. All of these studies confirm that blockade of chemokines results in a suppressive effect on glomerular leukocyte infiltration, proteinuria, and crescent formation in rodent models of glomerulonephritis, tubulointerstitial macrophage infiltration, and development of diabetic nephropathy. Furthermore, in type I and type II diabetic mouse models, deficiency of MCP-1/CCL2 not only ameliorates renal function but also reduces macrophage infiltration as well as renal fibrosis.

Like AKI, fractalkine/CX3CL1 is also detected in renal biopsies from patients with progressive kidney injury. Fractalkine/CX3CL1 and its receptor CX3CR1 are upregulated during renal injury in several mouse models, including experimental folic acid nephropathy, crescentic GN, and diabetic nephropathy. Treatment with a neutralizing antibody against fractalkine/CX3CL1 improves renal damage by blocking crescentic formation and macrophage infiltration in rat nephrotoxic nephritis. All these studies imply there might be an important role for CCL and CX3CL chemokines in chronic renal disease regardless of inciting events.

Role of Chemokines in T Cell Infiltration during Kidney Injury

Increasing evidence also suggests the functional importance of chemokines and their receptors in the recruitment of T cells into the kidney with chronic kidney disease. Fractalkine/CX3CR1 and its receptor CX3CR1 are expressed in CD3+ T cells and CD68+ macrophages infiltrating glomeruli and the interstitium. In a mouse model of crescentic glomerulonephritis, upregulation of renal CCR5 ligands (MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5) correlates with the recruitment of monocytes and T cells, suggesting a close link between the CCL chemokines and cell-mediated immune responses. This notion is further confirmed by finding that CCR5 null mice have an increased renal Th1 response. Because the CCR5 ligands (MIP-1α/CCL3 and RANTES/CCL5) also act through CCR1, blockade of CCR1, including the pharmacologic CCR1 antagonist, BX471, significantly reduces renal chemokine expression, T cell infiltration, and glomerular crescent formation in CCR5 null mice, indicating that increased renal leukocyte recruitment and subsequent tissue damage in nephritic mice depend on functional CCR1. Thus, CCR5 deficiency attenuates glomerulonephritis through enhanced CCL3/CCL5-CXCR1-driven recruitment of T cells and macrophages to the kidney. On the other hand, abrogation of Th1 signaling by deleting the T-bet gene, a transcription factor for Th1 cells, not only attenuates glomerular crescent formation and accumulation of CD4+ T cells and macrophages, but it also reduces intrarenal expression of RANTES/CCL5, Mig/CXCL9, and CXCR3. However, injection of Th1 cells in mice increases renal expression of MCP1/CCL2 and RANTES/CCL5, and causes progressive albuminuria along with crescent formation. Taken together, CCL chemokines participate in activating infiltrating cells related to Th1 immune responses during inflammatory renal injury.

Furthermore, CXCR3, which is highly expressed on Th1 CD4+ cells, is also responsible for the trafficking of Th1 cells into injured tissues because its expression correlates with elevated levels of urinary CXCR3+ CD4+ T cells, and upregulation of its ligand, intraperitoneally-10/CXCL10, in mouse models of systemic lupus erythematosus and anti-Thy-1 glomerulonephritis. CXCR3 deficiency reduces glomerular damage and T cell recruitment in crescentic glomerulonephritis.
Within injured kidneys.93 As CXCR3 is a trafficking receptor for both Th1 and Th17 cells, CXCR3 is one of the potential targets for therapy of T cell–mediated renal injury.

During inflammatory kidney injury, chemokines and their receptors also demonstrate a strong relationship with Tregs. As Tregs have anti-inflammatory properties, their presence is protective to renal injury.95–97 Tregs suppress production of MIP-1α/CCL3 by macrophages, which in turn protects against macrophage-dependent, lymphocyte-independent injury.95 Studies from CCR7 null mice demonstrate that CCR7 on Tregs is critical for homing of Tregs to lymphoid organs and guiding the Tregs to sites of antigen-specific activation.98–100 Importantly, adoptive transfer of CCR7+ Tregs, but not CCR7- Tregs, into CCR7 null mice restores Treg numbers in lymphoid organs and ameliorates disease.100 Finally, a recent study in a mouse model of nephrotic nephritis shows that CCR6 is expressed on renal FoxP3+ , CD4+ (Tregs), and IL-17–producing CD4+ (Th17) cells, but not on IFN-γ-producing Th1 cells.101 Upregulation of the CCR6 ligand, CCL20, also contributes to T cell recruitment, renal tissue injury, albuminuria, and loss of renal function. In contrast, deletion of CCR6 exacerbates renal injury and increases mortality among nephritic mice because of a reduction in infiltrating Tregs and Th17 cells with no affect on recruitment of Th1 cells.101 Furthermore, adoptive transfer of CCR6+ Tregs improves morphologic and functional renal injury, whereas transfer of Tregs from CCR6 null mice does not.101 These findings suggest that CCR6 mediates renal recruitment of both Tregs and Th17 cells and that the reduction of anti-inflammatory Tregs in the presence of a fully functional Th1 response aggravates renal progression in experimental glomerulonephritis.

In conclusion, results from human and experimental studies suggest that more than a single T cell subset is involved in renal injury.102 Understanding chemokine actions on cross-regulation of cell infiltration between Th1, Th17, and Treg cells should enable us to identify new therapeutic targets to treat renal injury.

CONCLUSION

In summary, evidence from experimental and clinical studies clearly demonstrates that chemokines are important regulators of leukocyte recruitment during kidney injury. Although interference with chemokine action holds great promises for the treatment of inflammatory renal diseases in experimental models, the chemotactic actions of chemokines in both initiation and progression of kidney diseases are much more promiscuous and complicated than we thought.1,2 Thus, much effort is required to further understand the mechanisms of chemokine function to develop innovative antichemokine therapies for human renal disease.

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DISCLOSURES

None.

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