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Roles of chemokines in renal ischemia/reperfusion injury

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TABLE OF CONTENTS

1. ABSTRACT

Ischemia-reperfusion injury (IR) is a common and an important clinical cause of renal disease, such as renal transplantation, renal artery stenosis and following shock from any cause. Inflammatory reaction after IR is regulated by various kinds of mediators. Chemokines are major mediators of the inflammation, and regulate pro-inflammatory cytokine and adhesion molecule expression, and leukocyte infiltration and activation. Chemokines are the key players of inflammation, angiogenesis and fibrosis. These inflammatory processes mediated by chemokines were observed in not only experimental animal models, but also in human renal diseases with ischemic injury. A number of challenges of chemokine targeted therapy is trying to prevent the ischemic injury, and will give some beneficial effect on the injury.

2. INTRODUCTION

Ischemia-reperfusion injury (IR) is a common clinical cause of renal disease. Unavoidable during renal transplantation, IR resulting in acute renal failure also occurs commonly in the context of renal artery stenosis and following shock from any cause. Like other pathologic conditions of the kidney, renal ischemia may ultimately progress to chronic, advanced kidney disease characterized by tubule and capillary loss as well as interstitial fibrosis. The underlying mechanisms appear to involve tubular epithelial cell necrosis and/or apoptosis, associated with marked leukocyte infiltration. Pro-inflammatory mechanisms resulting from IR in kidney have not yet been fully defined, but may provide opportunities for developing novel therapeutic approaches (1-3). This review focuses on the contribution of chemokines and chemokine receptors in coordinating inflammation in renal IR.

3. INDUCTION OF CHEMOKINES AFTER RENAL ISCHEMIA-REPERFUSION INJURY

The renal tubular epithelial cell is very sensitive to hypoxia, and may react to ischemic stress in at least three ways that may foster inflammatory responses. The first involves adenosine triphosphate (ATP) depression, which is an early event following oxygen deprivation due to ischemia. ATP depletion leads to inhibition of ATP-dependent transport pumps with loss of ion gradients that are normally maintained across intracellular organelles or cell membranes, resulting in mitochondrial swelling. Because the mitochondrial inner membrane has a larger surface area than its outer membrane, mitochondrial swelling results in outer membrane rupture, with release of mitochondrial intermembrane proteins. One of these, cytochrome c (4), activates an intracellular apoptotic signaling cascade involving cleavage of caspases 1 and 9. Caspase 1, or ICE (Interleukin-1 Converting Enzyme) enzymatically cleaves interleukin (IL)-1beta. Treatment with the caspase inhibitor, Z-Val-Ala-Asp (OMe)-CH2F, effectively prevents ischemia-induced renal tubular epithelial cell apoptosis, as well as ischemia-induced expression and release of the neutrophil-targeted chemokines KC (keratinocyte-induced chemoattractant) and MIP (macrophage inflammatory protein)-2 in the kidney. Accordingly, this agent inhibits neutrophil influx and functional impairment after renal IR (5).

A second factor that mediates ischemia-induced inflammation in the kidney is hypoxia inducible factor (HIF)-1. HIF-1 is a basic-helix-loop-helix heterodimeric transcription factor activated, as the name implies, by reduction in the partial pressure of oxygen. A conserved 28 bp hypoxia response element (HRE) can be found upstream of the transcription initiation site of HIF-1-dependent genes (6). HIF-1 is unstable under normoxic conditions because HIF-1 has a pO_2-dependent degradation domain that serves as a target for ubiquitination (7, 8). A study has shown that both IL-1 and tumor necrosis factor (TNF)-alpha increase HIF-1 DNA binding in hypoxic tubular epithelial cells *in vitro* (9), implying that the HIF-1 system may be enhanced under inflammatory conditions. As has been well-described, transactivation of HIF-1 transmits a hypoxic signal into pathophysiological responses, such as angiogenesis, erythropoiesis, vasomotor control, altered energy metabolism, as well as cell survival decisions by regulating a large cassette of target genes. In particular, many cytokines and growth factors use the HIF-1 signaling system for gene expression (10).

A third molecular switch linking ischemia to inflammation involves oxygen-derived free radicals, which have been implicated in cell signaling. In particular, hydrogen peroxide, which is a source of oxygen-derived free radicals after IR injury, has been reported to induce TNF-alpha production by activating p38 mitogen-activated protein kinase (MAPK) (11). Activation involves enhanced tyrosine phosphorylation, which can be blocked by dimethyl thiourea, a free radical scavenger (12). This pathway may also be relevant to chemokine expression since p38MAPK is in general a key pro-inflammatory mediator, acting in part by inducing chemokine/cytokine gene expression.

4. PRO-INFLAMMATORY CYTOKINES AUGMENT INFLAMMATION AFTER RENAL ISCHEMIA-REPERFUSION INJURY

Renal parenchymal cells, such as tubular epithelial cells, mesangial cells and endothelial cells, have the potential to produce various kinds of chemokines (e.g. CCL2/monocyte chemoattractant protein (MCP)-1, CCL5/regulated upon activation normal T cells expressed and secreted (RANTES), CXCL8/IL-8, CXCL1/growth-regulated oncogene (GRO), CXCL10/interferon-gamma Inducible Protein (IP)-10, CXCL2/3/MIP-2, etc) in response to stimulation with pro-inflammatory cytokines such as IL-1β, TNF-α, and interferon (IFN)-γ, immune complexes, and growth factors including platelet-derived growth factor and basic fibroblast growth factor (13). Midkine also enhances migration of inflammatory cells upon ischemic injury into the kidney, and may induce chemokine production and ischemic tissue damage (14, 15). Among chemokine/cytokine-producing cells in the kidney, the tubular epithelial cell appears to be the most sensitive to hypoxic conditions; moreover, tubular cell-derived cytokines/chemokines are thought to augment inflammatory processes in renal ischemia-reperfusion injury.

In addition to direct effects on tubule cells, pro-inflammatory cytokines and chemokines might amplify tubulo-interstitial inflammation after IR by inducing leukocyte infiltration. Moreover, leukocytes infiltrating the interstitium may also express chemokines in response to pro-inflammatory cytokine stimulation. For example, purified human blood monocytes have been reported to respond to oxidant stress mimicking IR with augmented production of CXCL8/IL-8 (16). Similarly, murine macrophages have been reported to upregulate expression of both TNF and CCL3/MIP-1alpha under anoxic conditions (16). These positive feedback loops involving cytokines/chemokines and inflammatory cells are key to augmenting inflammatory processes after IR.

5. INTRACELLULAR SIGNALING PATHWAYS LINKING ISCHEMIC INJURY TO CHEMOKINE PRODUCTION

Most pro-inflammatory chemokines, such as CXCL8/IL-8, CCL2/MCP-1 or CCL5/RANTES, are not expressed constitutively and require transcriptional activation by a variety of factors including nuclear factor (NF)-kappaB and activating protein (AP)-1 (17-19). Activation of these transcription factors requires phosphorylation by various kinases. In a positive feedback autocrine loop, inflammatory chemokines activate phosphorylation of p38 MAPK that contributes to activation of NF-kappaB and AP-1, which induce chemokine gene expression. Consistent with this, we found that pharmacologic inhibition of p38 MAPK significantly reduced inflammatory cytokine and chemokine production and prevented tissue destruction in a mouse model of renal

IR injury (20). Moreover, NF-kappaB decoy oligodeoxynucleotide treatment attenuated serum creatinine and BUN elevations as well as tubular necrosis with reduction of CCL2/MCP-1 expression in a rat model of renal IR injury (21). Thus, these intracellular mediators and the pathways they control may be good targets for therapeutic intervention in renal IR injury. However, further studies will be needed to clarify the importance and significance of each intracellular signaling cascade on cytokine and chemokine expression in renal IR injury.

6. CHEMOKINE EXPRESSION IN THE INJURED KIDNEY

Chemokines are able to activate many different cell types, including leukocytes and renal parenchymal cells. In addition to their chemotactic activity, chemokines have been implicated in the modulation of cell adhesion, phagocytosis, cytokine secretion, cell activation, cell proliferation, apoptosis, angiogenesis, proliferation and viral pathogenesis. They have been divided into four subfamilies according to the number and spacing of conserved cysteine residues in their sequences, and are classified into four main groups--CXC, CC, C, and CX3C—according to these cysteine patterns.

The CXC group contains the most important neutrophil-targeted chemokines, such as CXCL2/3/MIP-2 and KC in the mouse, and CXCL8/IL-8 in man, rabbit and other mammals. In the kidney these chemokines are upregulated in tubular epithelial cells after ischemic injury, and this results in marked neutrophil infiltration (5, 22-25). Antibodies to KC and MIP-2 inhibit neutrophil infiltration and restore renal function, limit tissue destruction and increase survival (25). CXCR2 is a specific receptor for KC and CXCL2/3MIP-2 in mouse and for CXCL8/IL-8 and related CXC chemokines in man and other mammals. CXCR2 was expressed in various organs, including kidney, after ischemic injury (24, 26-28). Injection with a novel low molecular weight inhibitor of human CXCR2 named repertaxin is effective in preventing neutrophil infiltration and renal dysfunction in a mouse model of renal IR injury (24). Repertaxin is now in clinical trial for lung transplantation, and would have potential for renal ischemic injury, including renal transplantation. Although CXCR2 on endothelial cells acts as an angiogenic factor, CXCR2 on leukocytes acts as an inflammatory mediator (29). Another CXC chemokine, stromal cell-derived factor (SDF)-1 (also known as CXCL12), is expressed constitutively in normal mouse kidney and is increased after IR injury, acting to recruit CD34-positive bone marrow-derived stem cells into the injured kidney (30). CXCL12/SDF-1 has also been reported to be expressed on endothelial cells in direct proportion to the local oxygen tension (31). The Th1 T lymphocyte-targeted chemokines CXCL10/IP-10 and monokine induced by IFN-gamma (Mig, CXCL9) have been detected, along with their shared receptor CXCR3, in the renal interstitium after mouse IR injury. After injury CXCR3-deficient mice show significantly lower serum creatinine levels, enhanced survival, and significantly less acute tubular necrosis and cellular infiltration than wild type controls (32). These data indicate that CXCL10/IP-10, CXCL9/Mig and CXCR3 may regulate inflammation after renal IR injury.

CC chemokines also participate in the pathogenesis of ischemic injury of the kidney. Expression of the monocyte-targeted CC chemokine CCL2/MCP-1 is induced in ischemic kidney, and this is associated with activation of NF-kappaB and monocyte infiltration (33). Both genetic deletion and pharmacological blockade of CCR2, the specific receptor for CCL2/MCP-1, have been reported to significantly reduce monocyte infiltration and tissue destruction after ischemic injury (20, 34, 35). As for KC and CXCL2/3/MIP-2, p-38 MAPK also plays a key role in CCL2/MCP-1 production. Moreover, expression of CCL2/MCP-1 is significantly and consistently enhanced in the absence of heme oxygenase (HO)-1 (36). Immunohistological studies indicate that the main resident cell producing CCL2/MCP-1 in the kidney after ischemic injury is located in the distal tubule (37). Macrophages are also a major source for MCP-1, and for the inflammatory cytokines TNF-alpha, IL-1 and IL-6 (38). IR injury also induces expression of the CC chemokines CCL3/MIP-1alpha and CCL5/RANTES (20, 39). The cognate receptors for these chemokines, CCR1 and CCR5, are also expressed in the ischemic kidney, and genetic deficiency of these receptors in mice significantly diminishes the number of leukocytes infiltrating the interstitium after IR injury (40).

CX3CL1/fractalkine and its unique receptor CX3CR1 are both upregulated after ischemic injury, and participate in the pathogenesis of renal fibrosis. The mechanism involves selective effects in the outer medulla, including accumulation of macrophages and expression of the macrophage and platelet-derived fibrogenic protein platelet-derived growth factor-B (41).

Together these studies reveal that inflammatory chemokines are expressed in the injured kidney and participate in inflammatory cell infiltration and activation, which participate in tissue destruction.

7. TEMPORAL AND SPATIAL ASPECTS OF LEUKOCYTE INFILTRATION IN RENAL IR INJURY

After IR injury, leukocyte subsets infiltrate the renal interstitium in a coordinated temporal sequence (42). Acute tubular necrosis occurs in the first phase of injury without significant cellular infiltration. This is followed by marked neutrophil infiltration. The process of tissue regeneration begins later and is associated with accumulation of macrophages and T cells and a paucity of neutrophils. This sequence is probably regulated in part by sequential expression of cell type-specific chemokines in the injured renal parenchyma. Each chemokine is thought to mediate key steps in the multistep mechanism of leukocyte trafficking, which involves three distinct interactions of blood leukocytes with endothelial cells on post-capillary venules: chemokine-independent selectin-mediated rolling, chemokine-dependent beta2-integrin-dependent firm arrest, and chemokine-dependent

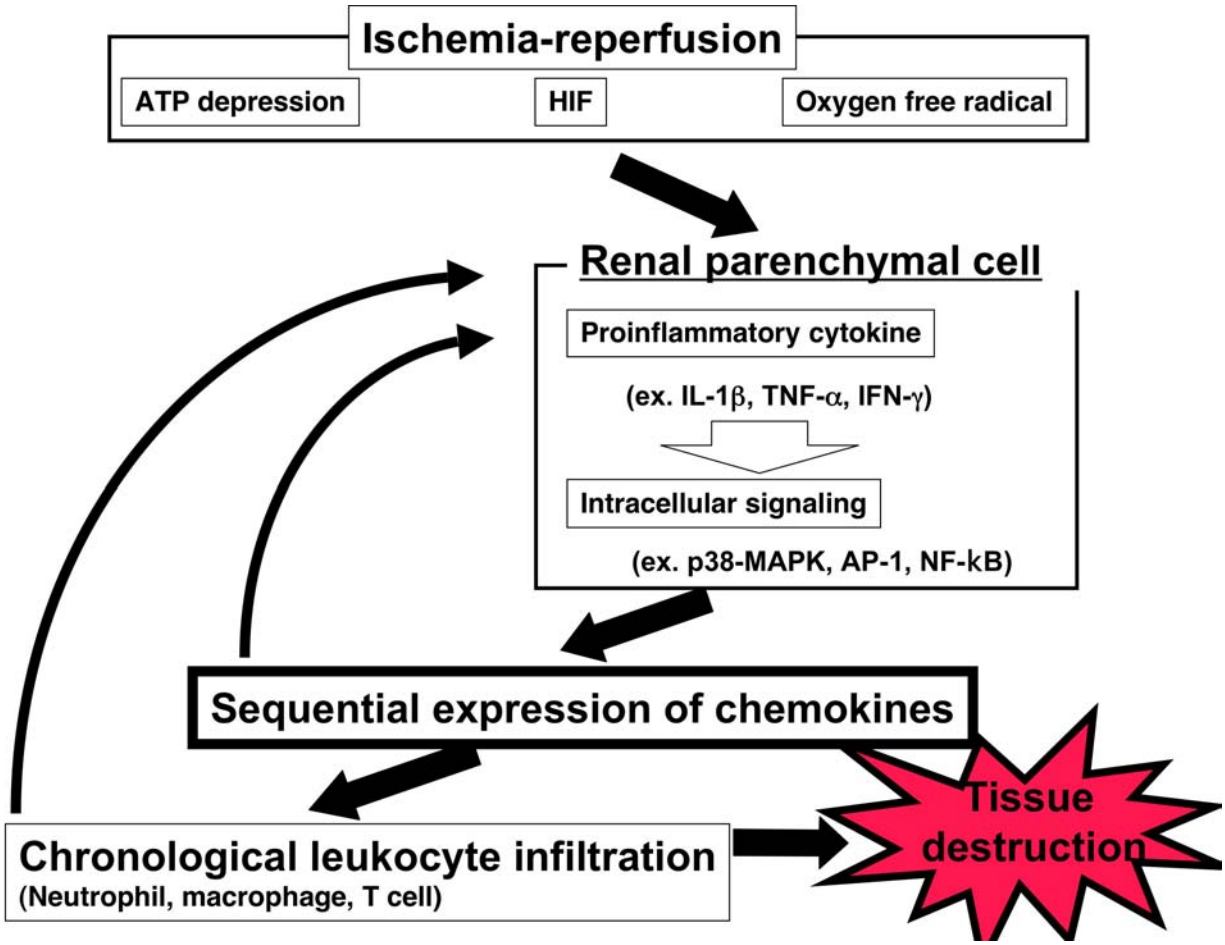

Figure 1. Inflammatory cascades in renal ischemia-reperfusion injury.Inflammatory cascades and feedback loops are key mediators of tissue destruction after renal ischemia-reperfusion injury. Sequential expression of chemokines is thought to be regulated by pro-inflammatory cytokine stimulation and leukocyte infiltration leading to tissue destruction.

. transendothelial migration (13, 43-45). Some chemokines, such as Growth-related oncogene (GRO) and fractalkine may mediate the initial firm adhesion, whereas CCL2/MCP-1 has been reported to be required for subsequent steps of leukocyte spreading and diapedesis (46, 47). Although precise mechanisms are unclear so far, each step during leukocyte transendothelial migration and extravasation might be regulated by unique sets of chemokines.

Consistent with this, we have found that tubular epithelial cells sequentially produce chemokines in response to pro-inflammatory cytokine stimulation, and that inflammatory cell subsets, such as neutrophils and macrophages, infiltrate injured tissue in a chronological manner in response to selective local chemokine expression (39).

8. CYTOKINES AND CHEMOKINES IN RENAL TRANSPLANTATION

Renal transplantation is one of the most important clinical situations in which the kidney is exposed to IR injury. In renal transplantation, immunological mechanisms leading to rejection are important, but non-immunological mechanisms such as IR are also important for long-term survival. Several factors, including chemokines, are upregulated within the first few hours without any apparent immunological reaction (48). Eventually chemokines and cytokines play important functional roles in acute allograft rejection in part by inducing mononuclear cell infiltration (49). The chemokines, CXCL8/IL-8, CXCL5/ENA-78, CCL2/MCP-1, CCL3/MIP-1alpha, CCL4/MIP-1beta, and CCL5/RANTES, have all been implicated in the pathogenesis of rejection, and should be considered as targets for prevention and treatment of allograft rejection (see Section 8). The study of associations of genetic polymorphisms in chemokine and chemokine receptor genes, such as those encoding CCL2/MCP-1, CCR2 and CCR5, with outcome after human renal transplantation suggests that these factors play a role in progression of renal destruction post-transplantation (50-52). Moreover, results from a rat model of transplantation have also supported a role for chemokines in renal tissue destruction (48, 53, 54). In addition to these chemokine receptors, a recent study has revealed a role for the Duffy antigen receptor for chemokines (DARC) in renal ischemic injury. DARC, which is present mainly on erythrocytes and

endothelial cells, is a promiscuous chemokine binding protein that does not appear to signal. DARC deficient mice exhibited no renal dysfunction and no postischemic neutrophil infiltration after ischemia-reperfusion injury. These data indicate that DARC predominantly exerts its effects by regulating neutrophil recruitment and subsequent acute renal failure (55). These human studies and animal models of transplantation together suggest that ischemia-induced cytokines and chemokines may participate in progression of tissue destruction and may be useful clinical markers for the intensity of tissue injury after transplantation.

9. Anti-inflammatory treatment approaches in renal IR injury

Although some vasoactive agents, such as dopamine, atrial natriuretic peptide and diuretics are clinically useful for acute renal failure after renal IR injury, these agents do not directly affect inflammation, which may be a major determinant of disease progression. Several experimental therapeutic approaches, such as leukocyte depletion, and anti-cytokine, anti-chemokine and anti-adhesion molecule therapies, have been tested with the aim of controlling renal inflammation after IR injury.

Caspase activation is one of the early events in the inflammatory response, driven by ATP depression in the context of ischemia, and has become a target for therapeutic development. Treatment with the caspase inhibitor, Z-Val-Ala-Asp (OMe)-CH2F, effectively prevents IR-induced renal apoptosis, KC and CXCL2/3MIP-2 up-regulation as well as neutrophil influx and functional impairment after renal ischemia-reperfusion (5). Complement activation products are also important inducers of chemokine expression, and selective antagonism of the C3a receptor significantly attenuates production of CXCL2/3MIP-2 and KC (22).

Intracellular signaling molecules are also target molecules for ischemic injury. Pretreatment of rats with NF-kappaB inhibitors inhibits CCL2/MCP-1 mRNA expression, and prevents tissue destruction after ischemia-reperfusion (33). Targeting p38 MAPK, a key factor regulating inflammatory chemokine expression, has been effective in significantly reducing chemokine expression and in attenuating leukocyte infiltration and tissue destruction (20).

Chemokines and chemokine receptors themselves are potentially important targets of anti-inflammatory therapy. We previously reported that neutralizing anti-IL-8 antibodies or anti-MCP-1 antibodies prevent experimental glomerulonephritis (56, 57). Moreover, specific inhibitors of chemokine receptors, such as vMIP-II, APO- or Met-RANTES, 7ND MCP-1, TAK-779 or propagermanium have been developed and tested successfully in various animal models, including experimental renal IR injury (34, 35, 58-62). These reports are encouraging, and add support for consideration of inflammatory chemokine receptors as targets for IR injury in the clinic.

The complex cascade of events induced by IR injury is likely to involve a balance of pro- and anti-inflammatory cytokines. In this regard, certain anti-inflammatory cytokines, working in part by blocking chemokine action, may be useful as therapeutic agents in IR injury. For example, osteopontin inhibits inducible nitric oxide synthase, and alpha-melanocyte-stimulating hormone inhibits both chemokine production (e.g. KC and CXCL8/IL-8) and adhesion molecule expression (e.g. ICAM-1) (63, 64). Insulin-like growth factor-1 and hepatocyte growth factor have also been reported to have beneficial effects in IR injury (53, 65, 66).

10. CONCLUSION

Chemokines are major mediators of inflammation in renal IR injury, acting at multiple levels including pro-inflammatory cytokine and adhesion molecule expression, and leukocyte infiltration and activation. Genetic experiments in mouse models have begun to dissect the key players and their specific roles at the level of inflammation, angiogenesis and fibrosis. A major challenge will be to expand this approach and to translate emerging results to human diseases in which renal IR injury occurs, with the goal of identifying novel targets for therapeutic intervention.

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