Chemokines in ischemia and reperfusion

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Summary
Chemokine signaling plays an important role in the post-ischemic inflammatory response. Overlapping pathways involving reactive oxygen intermediates, Toll-like receptor (TLR) activation, the complement cascade and the nuclear factor (NF)-kB system induce both CXC and CC chemokines in ischemic tissues. Reperfusion accentuates chemokine expression promoting an intense inflammatory reaction. ELR-containing CXC chemokines regulate neutrophil infiltration in the ischemic area, whereas CXCR3 ligands may mediate recruitment of Th1 cells. CC chemokines, on the other hand, induce mononuclear cell infiltration and macrophage activation. Evidence suggests that chemokine signaling mediates actions beyond leukocyte chemotaxis and activation, regulating angiogenesis and fibrous tissue deposition. Effective repair of ischemic tissue is dependent on a well-orchestrated cellular response and on timely induction and suppression of chemokines in a locally restricted manner. This manuscript reviews the evidence suggesting a role for chemokine-mediated effects in ischemia/reperfusion and discusses the potential significance of these interactions in injury and repair of ischemic tissues.

Keywords
Chemokine, ischemia, reperfusion, leukocyte, inflammation

Introduction
Ischemia is a state in which the arterial perfusion of an organ or tissue is insufficient to cater for its metabolic needs resulting in decreased oxygen tension within the cells and subsequent loss of oxidative phosphorylation and decreased generation of adenosine triphosphate (ATP). ATP depletion leads to failure of the sodium pump, loss of potassium, and accumulation of sodium and water producing acute cellular swelling. Tissues vary in their capacity to withstand a reduction in arterial perfusion. Neurons and cardiomyocytes are most susceptible to underperfusion and hypoxia, exhibiting irreversible changes after minutes of sustained ischemia. Cells dying by necrosis release their intracellular contents and initiate an intense inflammatory response by activating innate immune mechanisms. Toll-like receptor (TLR)-mediated pathways, complement activation and reactive oxygen species generation play a significant role in triggering the post-ischemic inflammatory response by activating the nuclear factor (NF)-kB system.

Reperfusion of the ischemic tissue after an episode of ischemia further accentuates the inflammatory reaction. Some investigators have suggested that reperfusion extends tissue injury due to release of free radicals, dysregulation of intracellular and mitochondrial calcium, microvascular dysfunction, or overzealous induction of injurious inflammatory mediators and infiltration with immune cells (1). Although there is experimental evidence supporting the concept of “reperfusion injury”, its significance remains a hotly debated issue. Early reperfusion has established beneficial effects in patients with acute myocardial infarction (MI) mostly due to enhanced survival of ischemic cardiomyocytes, but also possibly through activation of pathways that improve healing attenuating adverse remodeling. Thus, direct proof of an injurious effect of myocardial reperfusion in the clinical context is lacking.

Induction of chemokines is a prominent feature of the inflammatory response associated with ischemia and reperfusion in many tissues (2–4). A growing body of evidence suggests critical effects of selected members of the chemokine family in ischemic organs. The current review summarizes our understanding of the role of the chemokines in ischemic tissues and discusses the significance of chemokine-mediated interactions in regulating the cellular events associated with ischemia and reperfusion in various organs.
Mechanisms of chemokine induction in ischemic tissues

Chemokines are subdivided into CC, CXC, and CX3C families based on the number of amino acids between the first two cysteines. Lymphotactin (XCL1) is the only known chemokine containing only two cysteines corresponding to the second and fourth cysteines of other classes. CC chemokines are the most numerous and diverse family, including at least 25 ligands in humans. CXC chemokines are further classified according to the presence of the tripeptide motif glutamic acid-leucine-arginine (ELR) in the NH2 terminal region. From a functional standpoint chemokines can be divided broadly into two categories: homeostatic chemokines are constitutively expressed in certain tissues and may be responsible for basal leukocyte trafficking and formation of the fundamental architecture of lymphoid organs, whereas inducible chemokines are strongly upregulated by inflammatory or immune stimuli and actively participate in the inflammatory reactions by inducing leukocyte recruitment (5–7). Although this generalization has been challenged by studies demonstrating that certain chemokines, felt to be homeostatic (such as SDF-1) (8), are inducible upon immune activation, it offers valuable insight into the role of members of the chemokine family in pathological states. In ischemic tissues several overlapping pathways (including reactive oxygen, cytokines, the complement cascade, TLR-mediated mechanisms and the NF-kB system) can upregulate chemokines, leading to a rapid increase in their local concentration followed by leukocyte infiltration and an inflammatory response.

The role of reactive oxygen species (ROS) generation in triggering the chemokine response

ROS are atoms or molecules with unpaired electrons in their outer orbit. They are highly reactive entities and can participate in a variety of biochemical reactions (9). Molecular oxygen, O2, is diradical; each oxygen atom contains two unpaired electrons in its outermost shell. Full reduction of oxygen to water requires four electrons; however, the sequential donation of electrons to oxygen during this process can result in generation of ROS as intermediates. Under normal conditions O2 is reduced to H2O in the myocardium via two pathways: mitochondrial electron transport reduces 95% of O2 without any free radical intermediates (10). The remaining 5%, however, is reduced via the univalent pathway, producing free radicals. Donation of a single electron to molecular oxygen results in formation of the superoxide radical (O2-). Donation of a second electron yields peroxide, which then undergoes protonation to yield hydrogen peroxide (H2O2). Donation of a third electron results in production of the highly reactive hydroxyl radical.

ROS formation is markedly increased following ischemia and reperfusion. ROS react directly with cellular lipids, proteins and DNA, causing cell injury and death, and are critically involved in the oxidative burst reaction, which is essential for phagocyte function. In addition, ROS trigger cytokine and chemokine cascades through NF-kB activation (11–13). Evidence suggests that chemokine induction in models of brief myocardial ischemia and reperfusion is mediated mainly by reactive oxygen intermediates (14, 15). However, in myocardial infarcts cellular necrosis triggers additional chemokine-inducing pathways and the relative contribution of free radical generation remains unclear.

Cytokine-induced chemokine upregulation in ischemic tissues

Extensive evidence suggests that release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β stimulates chemokine synthesis in ischemic tissues. TNF-α-deficient mice undergoing experimental infarction protocols exhibit decreased chemokine and adhesion molecule expression suggesting an important role for TNF-α in mediating the post-infarction chemokine response (16). In the rat myocardium lipopolysaccharide-induced CXC chemokine (LIX) is expressed by resident myocardial cells during ischemia-reperfusion and is induced in cultured cardiomyocytes by oxidative stress or TNF-α via NF-κB activation (17). Induction of pulmonary CXCL5 following hepatic ischemia and reperfusion is dependent on liver-derived TNF-α release (18). In a model of hepatic ischemia and reperfusion defective IL-1 signaling resulted in markedly decreased chemokine upregulation and attenuated neutrophil infiltration (19). In addition to cytokine-mediated actions, performed inflammatory mediators, such as histamine and tryptase, released by resident mast cells may also contribute to chemokine upregulation in ischemic tissues (20, 21).

The complement cascade

The complement system is an important component of the innate immune response and a major effector in a variety of immunopathological diseases. The complement cascade is activated through three distinct mechanisms designated the classical, alternative and lectin pathways (22, 23). Numerous studies have indicated that ischemic injury activates the complement cascade (24). Hill and Ward (25) were the first to demonstrate that leukotactic activity in rat myocardial infarcts was in part due to C3 cleavage products. Complement activation may play an important role in mediating chemokine induction in ischemic tissues. Kilgore et al. (26) reported an attenuated CXCL8/IL-8 response accompanied by decreased neutrophil infiltration in C6-deficient rabbits, suggesting that the cytolytic membrane attack complex plays an important role in regulating expression of the chemokine in the infarct. Furthermore, administration of a specific C5a receptor antagonist abrogated up-regulation of CXC chemokines and reduced neutrophil infiltration by >50% in renal ischemia and reperfusion (27).

Activation of TLR-mediated pathways

The TLRs represent a family of pattern recognition receptors that serve to recognize molecular patterns associated with pathogens and, upon binding of their ligands, induce activation of several kinases and NF-κB. Endogenous ligands from damaged tissues, including heat shock proteins, hyaluronan and fibronectin, have the capacity to activate TLRs (28). Thus, in the absence of infection, endogenous “danger” signals activate TLR-mediated pathways initiating the immune response. To date, 12 members of the TLR family have been identified in mammals. TLR signaling appears to play an important role in chemokine upregulation in ischemic organs. The rapid breakdown of extracellular matrix in ischemic tissues may result in accumulation of hyaluronan frag-
ments, which are capable of inducing chemokine synthesis in macrophages (29) and endothelial cells (30). Endothelial chemokine upregulation by hyaluronan fragments is TLR4-dependent, as shown by experiments using TLR4-blocking antibody and TLR4-deficient mice (31).

TLR4 was required for chemokine induction in hepatic ischemia and reperfusion (32). In addition, TLR4-deficient mice had decreased infarct size and suppressed inflammation following MI (33). Ligand activation of TLR2, TLR4 and TLR5 initiates an NF-κB-dependent inflammatory response inducing cardiomyocyte expression of the CXC chemokines keratinocyte-derived chemokine (KC) and macrophage inflammatory protein (MIP)-2 (34).

### The NF-κB system

A critical element in the regulation of cytokine, chemokine and adhesion molecule expression in ischemic tissues involves the complex formed by NF-κB and IκB (35). NF-κB is activated by a large number of agents, including cytokines (such as TNF-α and IL-1β) and free radicals. The genes regulated by the NF-κB family of transcription factors are diverse and include those involved in the inflammatory response (such as chemokines and cytokines), cell adhesion and growth control (36). In addition, experimental studies have suggested that the NF-κB pathway may also mediate cytoprotective responses (37). Transgenic mice harboring cardiac-restricted expression of a mutated IκBα protein that prevents nuclear translocation of NF-κB in cardiac myocytes had larger infarcts and significantly enhanced myocyte apoptosis in a model of permanent coronary occlusion (38). In addition, retroinfusion of NF-κB oligonucleotides in a porcine model of myocardial ischemia reduced infarct size and improved functional reserve of the area at risk, providing post-ischemic cardioprotection (39). Furthermore, it has been suggested that NF-κB activation in leukocytes during the resolution phase of the inflammatory process results in upregulation of anti-inflammatory genes and induces leukocyte apoptosis (40). Activation of the NF-κB signaling cascade in multiple parallel processes involving various cell types, implicated in the response to ischemia, further complicates understanding of its role in ischemic tissues.

The role of the CXC chemokines in ischemia and reperfusion (Fig. 1)

**ELR+ CXC chemokines regulate neutrophil recruitment in ischemic tissues**

CXC chemokines play an important role in regulating neutrophil chemotaxis and activation in ischemic tissues (41). The prototypic CXC chemokine IL-8/CXCL8 was purified as a monocyte-derived factor that attracts neutrophils, but not monocytes, in Boyden chamber assays (42). Several other CXC chemokines are also potent neutrophil chemoattractants and structure/activity analyses showed that this property depends on the presence of the tripeptide ELR (glutamic acid – leucine – arginine) motif, between the N-terminus and the first cysteine (43, 44).

IL-8 upregulation has been documented in animal models of myocardial, pulmonary and cerebral ischemia and reperfusion (45–48). In a canine model of reperfused infarction, IL-8 synthesis was accentuated by reperfusion and was localized in the inflammatory infiltrate of the infarct border zone, as well as in small veins in the same area (45). Recombinant canine IL-8 markedly increased adhesion of neutrophils to isolated canine cardiac myocytes (45), suggesting a potential role in neutrophil-mediated myocardial injury. Several studies using antibody neutralization have supported an important role for IL-8 in mediating neutrophil-induced injury in ischemic and reperfused tissues. Administration of a neutralizing anti-IL-8 antibody prevented neutrophil infiltration and tissue injury in a model of pulmonary ischemia/reperfusion (47) and reduced infarct size and brain edema in a cerebral ischemia/reperfusion (48). Furthermore, IL-8 neutralization significantly reduced the degree of necrosis in a rabbit model of myocardial ischemia and reperfusion without affecting neutrophil infiltration (49). Recent studies suggested that IL-8 may have effects beyond its neutrophil chemotactic actions inducing homing of endothelial progenitor cells and stimulating angiogenesis in the ischemic heart (50). Unfortunately, elucidating the role of IL-8 in ischemic tissues using knockout and transgenic animals is hampered by the absence of an IL-8 homolog in rodents.

Considerably less is known regarding the role of other ELR-containing CXC chemokines in myocardial infarcts. Growth-re-
lated oncogene (GRO)-α/CXCL1 was so named because of its initial description as the product of a gene differentially expressed in transformed hamster cells that had suffered loss of growth control (51). Independently, its murine homolog was cloned in a differential screening experiment as the platelet-derived growth factor (PDGF)-inducible KC gene (52).

GRO-α/KC, a potent neutrophil chemoattractant, is induced in a rat model of experimental MI (17), and in mouse models of colonic and renal ischemia (53, 54). GRO-α/KC neutralization significantly inhibited neutrophil infiltration in the ischemic kidney during reperfusion attenuating renal dysfunction (55) and attenuated hepatocellular injury in a model of hepatic ischemia/reperfusion (56). GRO-β/CXCL2 and GRO-γ/CXCL3 are closely related proteins that are also potent neutrophil chemoattractants; their potential role in mediating leukocyte infiltration and injury in ischemic tissues has not been studied. Epithelial neutrophil activating protein (ENA-78/CXCL5) is another ELR-containing CXC chemokine that exhibits similarities with the GROs. ENA-78 is an important mediator in neutrophil recruitment and lung injury induced by hepatic ischemia and reperfusion (18). Rodents lack an IL-8 homolog but possess other ELR positive CXC chemokines that signal via CXCR2 inducing neutrophil chemotaxis and activation, namely CINC-1 and MIP-2. Experimental studies suggested a key role for MIP-2 in regulating neutrophil infiltration in renal (55), and hepatic ischemia and reperfusion (56).

Two specific receptors for the ELR+ CXC chemokines, CXCR1 and CXCR2, have been identified on the cellular surface. Deficiency of CXCR2, the main receptor for the ELR-containing CXC chemokines in mice, resulted in significantly decreased inflammatory leukocyte recruitment in murine infarcts, suggesting a crucial role for these chemokines in inflammatory cell infiltration (57). Furthermore, CXCR2 inhibition using antibodies, or the low-molecular-weight inhibitor repertaxin attenuated ischemia/reperfusion injury in various experimental models (58, 59). On the other hand, experiments using a Langendorff preparation indicated protective effects of CXCR2 signalling on myocardial viability (57). The molecular basis for the presumed direct effects of CXCR2 signaling on cardiomyocytes remains unclear.

The role of the ELR-negative CXC chemokines in ischemia and reperfusion

In contrast to the potent neutrophil chemotactic actions of ELR+ CXC chemokines, CXC chemokines that do not contain the ELR motif do not attract granulocytes but may play a role in post-ischemic inflammation by inducing Th1-cell infiltration. The CXCR3 receptor is predominantly expressed on activated Th1 cells and binds three ELR negative CXC chemokines: monokine induced by γ-interferon (MIG/CXCL9), interferon-γ inducible protein (IP)-10/CXCL10 and interferon-inducible T-cell alpha chemoattractant (ITAC/CXCL11). Compared with wildtype mice, CXCR3 null animals exhibited attenuated renal dysfunction following kidney ischemia and reperfusion. The protective effect of CXCR3 deficiency was due to decreased recruitment of Th1 cells in the ischemic kidney (60).

In addition to their potential role in regulating post-ischemic inflammation, certain ELR-negative CXC chemokines (such as platelet factor 4 [PF4/CXCL4], IP-10/CXCL10, and MIG/ CXCL9) also exhibit robust angiostatic effects. In contrast to the angiogenic actions of ELR+ CXC chemokines, members of the ELR negative subfamily not only failed to induce significant in-vitro endothelial cell chemotaxis or in-vivo corneal neovascularization, but were found to be potent angiostatic factors in the presence of IL-8 or basic fibroblast growth factor (bFGF) (61, 62). In addition, IP-10/CXCL10 may have direct inhibitory effects on fibroblast migration (63), serving as an antifibrotic agent. However, the significance of these properties in the biology of tissue ischemia remains unknown. A recent investigation demonstrated that CXCR3 null mice had impaired angiogenesis in a model of hindlimb ischemia, associated with decreased T-cell and macrophage infiltration in the ischemic area (64). This finding suggested that CXCR3 ligands may enhance angiogenesis through recruitment of inflammatory leukocytes capable of producing angiogenic mediators.

Investigations from our laboratory are exploring the role of CXCR3 ligands in the ischemic myocardium. Using a canine model of reperfused infarction we demonstrated a marked transient upregulation of IP-10 in ischemic myocardial segments (65). IP-10 mRNA expression was downregulated following 24 hours of reperfusion, whereas IL-8 message levels remained high. IP-10 mRNA and protein was localized in the microvascular endothelium of ischemic myocardial segments (65). In-vitro experiments demonstrated that TNF-α, which is released early after myocardial ischemia (20), markedly upregulates IP-10 expression in canine venous endothelial cells (65, 66). In order to investigate the mechanisms of IP-10 downregulation after 24 hours of reperfusion, we studied the effects of IL-10 and transforming growth factor (TGF)-β, both present in the ischemic myocardium (67, 68) in regulating cytokine-induced IP-10 expression. Our experiments demonstrated that TGF-β and not IL-10 is capable of suppressing TNF-α-mediated IP-10 upregulation in canine endothelial cells. Studies using IP-10 and CXCR3 null mice will elucidate the role of CXCR3 and its ligands in the ischemic myocardium.

It is likely that CXCR3 ligands affect several distinct pathways in ischemic tissues. First, CXCR3 signaling may induce recruitment of specific lymphocyte subsets promoting tissue injury. Second, CXCR3 ligands may indirectly regulate infiltration with monocyte/macrophages capable of producing angiogenic mediators. Third, direct angiostatic and anti-fibrotic actions of the CXCR3 ligand IP-10 may play an important role in the healing process. The early transient induction of IP-10 in the ischemic myocardium may serve to prevent premature wound angiogenesis and fibrous tissue deposition in the infarct, until the injured myocardium has been cleared from dead cells and debris by infiltrating phagocytes, and a fibrin-rich provisional matrix is formed in order to support ingrowth of granulation tissue. The relative significance of each one of these pathways following an ischemic insult remains unknown.

Stromal cell-derived factor (SDF)-1/CXCL12 is a non-ELR-containing CXC chemokine with a critical role in cardiovascular development (69) and angiogenesis (70, 71), mediated through interactions with its receptor CXCR4. In addition, SDF-1 induces chemotaxis of CD34+ progenitors (72) and primitive hematopoietic cells (73) and controls many aspects of stem cell function.
Chemokines in vascular biology

The role of CC chemokines in ischemia and reperfusion (Fig. 2)

CC chemokines are functionally diverse and their names more often reflect historical accidents of their cloning or isolation than their predominant functions (42). One of the best-studied CC chemokines, MCP-1/CCL2, is a potent chemoattractant for monocytes, T cells and NK cells and has been implicated in diseases characterized by monocyte-rich infiltrates (81, 82). Its expression and functional significance have been documented in a wide variety of disease processes, such as atherosclerosis (83), multiple sclerosis (84), rheumatoid arthritis (85), and nephritis (86). Extensive evidence suggests a key role for the MCP-1/CCR2 axis in ischemic tissues. MCP-1/CCL2 upregulation has been demonstrated in experimental models of myocardial infarction (87, 88), renal (89) and cerebral ischemia (90).

In a canine model of myocardial ischemia/reperfusion induction of MCP-1 mRNA occurred only in ischemic segments within the first hour of reperfusion, peaked at three hours, and persisted throughout the first two days of reperfusion. In the absence of reperfusion, MCP-1 induction was significantly lower (91). MCP-1 was localized on infiltrating leukocytes and venular endothelial cells. Additional experiments suggested that MCP-1 may be a major factor responsible for mononuclear cell recruitment into the ischemic myocardium during the first five hours of reperfusion (68). In a rat model of experimental MI, administration of a neutralizing antibody to MCP-1 significantly reduced infarct size decreasing adhesion molecule expression and macrophage infiltration (92). The development of mice with genetic disruption of MCP-1 and its ligand CCR2 significantly contributed to our understanding of the role of the MCP-1/CCR2 axis in ischemic tissues. CCR2 null mice exhibited attenuated tubular necrosis and decreased macrophage infiltration following renal ischemia and reperfusion (89). Recent investigations from our laboratory provided new insight into the role of MCP-1 in the ischemic myocardium. MCP-1 +/- mice had decreased and delayed macrophage infiltration in the healing infarct and exhibited delayed replacement of injured cardiomyocytes with granulation tissue. MCP-1 +/- infarcts had decreased mRNA expression of the cytokines TNF-α, IL-1β, TGF-β, and IL-10 and demonstrated defective macrophage differentiation evidenced by decreased osteopontin (OPN)-1 expression. MCP-1 deficiency diminished myofibroblast accumulation but did not significantly affect infarct angiogenesis. Despite showing delayed phagocytic removal of dead cardiomyocytes, MCP-1 +/- mice had attenuated left ventricular remodeling, but similar infarct size when compared with wild-type animals. MCP-1 antibody inhibition resulted in defects comparable with the pathological findings noted in infarcted MCP-1 +/- animals without an effect on macrophage recruitment (93).

Our findings indicated that MCP-1 has important effects on macrophage recruitment and activation, cytokine synthesis and myofibroblast accumulation in healing infarcts. The role of MCP-1 extends beyond its leukotactic effects modulating angiogenesis and fibrosis and inducing apoptosis on ischemic cardiomyocytes. MIP-1α and MIP-1β are also induced in ischemic tissues and have mononuclear cell chemoattractant properties. However, their role in regulating post-ischemic inflammation remains poorly understood. F, fibroblast; E, endothelial cell; M, mononuclear cell; CM, cardiomyocyte.

Figure 2: The role of CC chemokines in ischemic tissues. MCP-1/CCL2 plays a key role in regulating mononuclear cell recruitment and activation. In addition, MCP-1 may exert actions beyond its leukotactic effects modulating angiogenesis and fibrosis and inducing apoptosis on ischemic cardiomyocytes. MIP-1α and MIP-1β are also induced in ischemic tissues and have mononuclear cell chemoattractant properties. However, their role in regulating post-ischemic inflammation remains poorly understood. F, fibroblast; E, endothelial cell; M, mononuclear cell; CM, cardiomyocyte.

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lations have been identified that circulate in approximately equal numbers (94); a CCR2-positive subset with low expression of the fractalkine receptor CX3CR1 (CX3CR1 lo), preferentially recruited in inflammatory processes, and a CCR2-negative subpopulation comprised of cells with high level CX3CR1 expression (CX3CR1 hi), that home to normal tissues and become resident macrophages. In the absence of MCP-1, the “selection advantage” of the CCR2-expressing population may be lost, resulting in decreased and delayed infiltration of the infarct with both subsets of monocytes in equal numbers. CX3CR1 hi monocytes may exhibit decreased cytokine expression and/or phagocytic activity upon stimulation with pro-inflammatory mediators. These concepts appear to be highly relevant in human pathobiology. Subsets of human peripheral blood monocytes with distinct chemokine receptor profiles have been identified. CD14+CD16+ monocytes express lower CCR2 but higher CCR5 levels; in contrast CD14++ monocytes exhibit high CCR2 and low CCR5 expression (95). Polarized CCR2 expression is accompanied by differential chemotactic responsiveness to MCP-1 (95).

2) In addition to its chemotactic properties, MCP-1 may also directly modulate macrophage differentiation, phagocytic activity and cytokine expression. Previous investigations indicated that MCP-1 induces monocyte IL-1 (96) and IL-6 synthesis (97) and that it may be involved in differentiation of monocytes into foam macrophages (98).

3) The reduced myofibroblast density in MCP-1 null infarcts may result from decreased proliferative activity of resident fibroblasts or impaired recruitment of fibroblast progenitor cells, capable of differentiating into fibroblasts. The significance of these cells in the infarcted myocardium remains unknown.

The key role of MCP-1 signaling in the pathogenesis of post-infarction remodeling was also suggested by experiments using mice with genetic disruption of CCR2, the primary receptor for MCP-1 (99). CCR2 null mice had decreased infiltration with macrophages and exhibited attenuated ventricular dilation following MI. CCR2 absence was associated with markedly decreased metalloproteinase expression and lower gelatinolytic activity in the infarcted ventricle. Attenuated matrix degradation may explain, at least in part, the protection from the development of adverse remodeling noted in CCR2 null animals.

A recently published investigation demonstrated that transgenic mice with cardiac overexpression of MCP-1 had enhanced macrophage infiltration, and increased accumulation of myofibroblasts and endothelial cells in the infarcted myocardium (100). Surprisingly, these findings were associated with attenuated systolic dysfunction. Although these cellular effects of MCP-1 overexpression are consistent with the biological actions of the chemokine, the significant cardiac inflammation and fibrosis noted in MCP-1 overexpressing hearts in the absence of injury make interpretation of the findings difficult.

MCP-3, another potent mononuclear cell chemotactant, is also transiently induced in the ischemic rat brain (101) and in mouse myocardial infarcts (102). Transplantation of MCP-3-expressing cardiac fibroblasts into the infarct border zone one month after coronary ligation resulted in enhanced homing of injected mesenchymal stem cells in the infarcted myocardium (102). However, the exact role of MCP-3-mediated interactions in ischemic tissues remains poorly understood.

Macrophage inflammatory protein (MIP)-1α and MIP-1β are also mononuclear cell chemoattractants, although less efficient than MCP-1 (103). A robust induction of MIP-1α and MIP-1β was noted in experimental models of myocardial (104) and cerebral (105) ischemia and reperfusion. However, the importance of these chemokines in the pathogenesis of ischemic injury and in the cellular events involved in tissue repair remains poorly understood. An experimental study demonstrated that MIP-1α neutralization significantly reduced vascular permeability in a model of pulmonary ischemia and reperfusion (106). Studies using animals with targeted disruption of the MIP-1α and MIP-1β genes in ischemia/reperfusion models are lacking.

The cDNA encoding RANTES (Regulated upon Activation, Normal T-cell Expressed and Secreted) was isolated in a T-versus B-lymphocyte differential screen, and found to be inducible by mitogens or antigen in a variety of T-cell lines and circulating...

**Figure 3:** The role of the chemokines in the ischemic myocardium. Myocardial ischemia potently induces expression of CXC and CC chemokines that critically regulate the inflammatory response. The post-infarction inflammatory reaction is closely intertwined with cardiac remodeling, a complex process that results in dilation of the ventricle and is associated with dysfunction and adverse events.
lymphocytes (107). Systemic release of RANTES, an important chemoattractant for monocytes, eosinophils, and specific subsets of T cells (108) was found in the serum from patients with acute MI (109); however, local induction of this chemokine was not observed in the ischemic kidney (110) and heart (unpublished observation).

Fractalkine signaling in ischemia/reperfusion

Fractalkine/CX3CL1, the only member of the CX3C chemokine subfamily, is expressed on NK cells, monocytes and T cells and functions as a leukocyte chemoattractant and as an adhesion molecule. Few studies have explored the role of fractalkine and its receptor CX3CR1 in ischemic injury. Fractalkine is constitutively expressed in the kidney, but its expression is redistributed to the outer medulla following renal ischemia and reperfusion. Mice with genetic disruption of the fractalkine receptor CX3CR1 and animals injected with neutralizing anti-CX3CR1 antibody exhibited attenuated fibrosis following renal ischemia and reperfusion, suggesting that CX3CR1 signaling regulates macrophage accumulation and fibrous tissue deposition in the ischemic and reperfused kidney (111). In addition, fractalkine null mice had decreased infarct size in a model of cerebral ischemia and reperfusion (112).

The significance of chemokine-mediated interactions in ischemic organs

Chemokines in the ischemic myocardium (Fig. 3)

Minutes after the onset of myocardial ischemia reversible ultrastructural cardiomyocyte changes appear, including cellular and mitochondrial swelling and glycogen depletion. Irreversible cardiomyocyte injury, evidenced by sarcolemmal disruption and the presence of small amorphous densities in the mitochondria, develop after 20–40 minutes of sustained ischemia (113). Cells dying by necrosis release their intracellular contents and initiate an intense inflammatory response by activating innate immune mechanisms. The inflammatory cascade serves to clear the infarct from dead cells and matrix debris, but also results in healing and replacement of the damaged tissue with scar. Thus, cardiac repair following MI is closely intertwined with the inflammatory response. Infarct healing can be divided into three overlapping phases: the inflammatory phase, the proliferative phase and the maturation phase. During the inflammatory phase activation of chemokine and cytokine cascades results in recruitment of leukocytes into the infarcted area. Neutrophils and macrophages clear the wound from dead cells and matrix debris. Activated macrophages release cytokines and growth factors leading to formation of granulation tissue. Induction of pro-inflammatory mediators is followed by resolution of the neutrophilic infiltrate and repression of cytokine and chemokine synthesis, while fibroblasts and endothelial cells proliferate (114–116). During the proliferative phase of healing, activated myofibroblasts produce extracellular matrix proteins and an extensive microvascular network is formed. Maturation of the scar follows: fibroblasts and vascular cells undergo apoptosis and a collagen-based scar is formed (117). Infarct healing results in profound changes in ventricular architecture and geometry, also referred to as “ventricular remodeling”. The molecular and cellular changes associated with ventricular remodeling affect both the cardiomyocytes and interstitial cells and manifest clinically as increased ventricular size, altered shape of the ventricle and worsened cardiac function. Remodeling is linked to heart failure progression and is associated with poor prognosis following MI. Ventricular dilation following MI is an important predictor of mortality (118) and adverse cardiac events, including the development of heart failure and ventricular arrhythmias.

Chemokine induction is a prominent feature of the post-infarction inflammatory response. ELR containing CXC chemokines regulate neutrophil infiltration in the infarcted myocardium (2). However, the potential role of neutrophils in extending injury in the ischemic myocardium remains controversial. The role of ELR-negative CXC chemokines in myocardial ischemia is less well established. IP-10 is transiently induced in the ischemic myocardium and may serve to prevent premature wound angiogenesis and fibrous tissue deposition in the infarct, until a provisional matrix network is formed in order to support neovessel formation and fibroblast infiltration. SDF-1, on the other hand, may induce recruitment of progenitor cells in the ischemic heart.

CC chemokines mediate mononuclear cell infiltration in the infarct. Animals with genetic disruption of MCP-1 and its main receptor, CCR2, exhibit decreased post-infarction remodeling (99). In addition, anti-MCP-1 gene therapy (119) significantly reduced ventricular dilation in a mouse model. These findings suggest that MCP-1 may be a novel pharmacologic target in patients with acute MI. However, a word of caution should be raised: absence of MCP-1 results in attenuated post-infarction left ventricular remodeling, at the expense of a prolonged inflammatory phase and delayed replacement of injured cardiomyocytes with granulation tissue. Defective MCP-1 signaling results in impaired macrophage activation, defective phagocytosis of injured cardiomyocytes and delayed granulation tissue formation. It should be noted that timely repression of chemokine expression after the inflammatory phase is important for resolution of the inflammatory infiltrate (104) and plays an important role in regulating infarct healing (116, 120, 121).

In addition to their role in regulating the cellular events following MI, CC chemokines are also induced after brief ischemic insults that do not result in cardiomyocyte necrosis (14, 15). Brief episodes of repetitive ischemia and reperfusion induce marked MCP-1 upregulation in the mouse myocardium in the absence of significant cardiomyocyte death. Chemokine-driven inflammation in this model results in the development of extensive interstitial fibrosis and left ventricular dysfunction (122). Both MCP-1 gene disruption and inhibition with a neutralizing antibody protected the myocardium from the development of interstitial fibrosis and attenuated left ventricular dysfunction (123). In the cardiomyopathic heart MCP-1 may mediate its profibrotic effects through several distinct mechanisms. First, mononuclear cells chemotactically attracted through MCP-1/CCR2 signaling may be an important source of fibrogenic mediators, such as TGF-β and fibroblast growth factors. Second, MCP-1 may directly modulate fibroblast phenotype and activity. Third, MCP-1 may be an important mediator in the recruitment
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Chemokines in ischemia/reperfusion of fibroblast progenitor cells (124). Recent investigations indicated that MCP-1/CCR2 signaling is important for recruitment of fibrocytes to the alveolar space after fibrotic injury in the lung (125). The significance of MCP-1 signaling in mediating the development of cardiac fibrosis and ischemic cardiomyopathy was first suggested by our investigations using samples obtained from dysfunctional myocardial segments from patients with ischemic cardiomyopathy undergoing aortocoronary bypass surgery. Myocardial segments with significant dysfunction showed increased MCP-1 expression and local leukocyte infiltration (126). Furthermore, segments with recovery of function after surgical revascularization exhibited higher MCP-1 levels and enhanced inflammatory activity in comparison to segments with irreversible dysfunction. In contrast, persistently dysfunctional myocardium exhibited decreased inflammatory activity and enhanced collagen deposition (127). These findings suggested that the development of ischemic cardiomyopathy is a continuous process: at an early stage brief episodes of ischemia may induce an inflammatory response activating fibrogenic pathways. However, prolonged activation of ischemia-induced inflammation may trigger inhibitory mechanisms (such as TGF-β activation) that suppress pro-inflammatory mediator synthesis, while inducing synthesis of genes associated with fibrosis (2). The involvement of MCP-1 signaling in the pathogenesis of chronic ischemic heart disease was also supported by recent findings demonstrating markedly enhanced expression of a novel transcription factor, termed MCP-induced protein (MCPIP), that causes cell death in patients with ischemic cardiomyopathy (128, 129). Thus MCP-1 induction in the chronically ischemic heart may also activate pro-apoptotic pathways, in the absence of MI.

Chemokines in renal ischemia and reperfusion

Acute ischemia of the kidney is a major cause of renal failure occurring most commonly in the setting of renal artery stenosis, renal transplantation and shock due to hemorrhage or sepsis. Tissue injury following renal ischemia and reperfusion may be due at least in part to activation of inflammatory pathways and recruitment of leukocytes. Induction of ELR-containing CXC chemokines, such as IL-8/CXCL8, may play an important role in neutrophil recruitment in the ischemic kidney mediating tissue injury through the release of cytokines, free radical intermediates and tissue degrading proteases (55, 130). CXCR3 ligands, on the other hand, regulate recruitment of Th1 cells to the ischemic kidney inducing further tubular damage.

The MCP-1/CCR2 axis is also important for acute tubular necrosis in the ischemic kidney by regulating infiltration and activation of macrophages (89). Development of interstitial fibrosis is one of the major sequelae of renal ischemia and reperfusion. Signaling through the fractalkine receptor CX3CR1 appears to contribute to fibrogenesis in renal ischemia and reperfusion (111).

Chemokines in the ischemic brain

Cerebral ischemia is associated with marked induction of both CXC and CC chemokines resulting in extensive leukocyte infiltration in the ischemic brain. Neutrophil infiltration may increase cerebral edema inducing injury in the ischemic area. Strategies inhibiting neutrophil chemoattractant CXC chemokines significantly attenuated brain edema and reduced infarct volume in experimental models of cerebral ischemia and reperfusion (48). CC chemokine and fractalkine signaling may also augment post-ischemic injury in the brain.

Chemokine signaling in hepatic ischemia and reperfusion

The early phase of injury induced by hepatic ischemia and reperfusion is a free radical-mediated event (131, 132). The later phase of injury depends on neutrophil extravasation and requires a parenchymal chemotactic signal (3). Synthesis of ELR-positive chemokines by hepatocytes provides one of the key signals for neutrophil recruitment in the ischemic liver. Necrosis of ischemic hepatocytes results in activation of the complement cascade and triggers reactive oxygen generation and cytokine synthesis by Kupffer cells further accentuating the inflammatory reaction. One of the striking consequences of liver injury is the associated pulmonary dysfunction, which is linked with the release of hepatic-derived cytokines and involves pulmonary chemokine induction that triggers neutrophil sequestration in the lung (18).

Concluding remarks

Chemokine signaling regulates many steps of the inflammatory response in ischemic tissues. ELR-containing CXC chemokines play a key role in neutrophil recruitment in the ischemic area, whereas CC chemokines attract and activate monocyte/macrophages. However, chemokine signaling mediates actions beyond leukocyte chemotaxis, inducing angiogenic and profibrotic effects. Although the cellular effects of chemokine signaling have similarities in various ischemic organs, the clinical implications of these actions are dependent on the context and the unique characteristics of the local environment. For example in the infarcted heart, the chemokine response is an important regulator of cardiac remodeling affecting systolic function by modulating the geometric characteristics of the ventricle. In all tissues effective healing depends on a well-orchestrated cellular response and on timely induction and suppression of specific mediators in a locally restricted manner. Repression of chemokine synthesis after a transient early peak is important for optimal tissue repair. Thus, interventions targeting the chemokine system should take into account these temporal and spatial parameters.

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Fragogiannis: Chemokines in ischemia/reperfusion


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