Chemokines in Cardiovascular Diseases: From Atherosclerosis and Heart Failure to Allograft Arteriopathy

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Chemokines are a superfamily of structurally related cytokines that mediate leukocyte attraction and migration during inflammatory and immune responses. They are divided into 4 groups (CXC, CX3C, CC, and C), on the basis of their amino acid sequence in relation to their cysteine moieties. Chemokines have been shown to play a pathogenic role in several inflammatory disorders and have been implicated in the pathogenesis of several cardiovascular diseases such as congestive heart failure, atherosclerosis, myocarditis, and ischemia-reperfusion injury. Recently, an important role for chemokines has also been demonstrated in the pathogenesis of acute rejection and allograft vasculopathy following cardiac transplantation. Chemokine antagonism may represent a potential novel therapeutic strategy to attenuate the inflammatory response of several disease processes.

Introduction

Chemokines are small peptides (8-11 KD) that mediate the activation and recruitment of specific subsets of leukocytes in the inflammatory and immune response. More than 50 chemokines, and at least 18 different chemokine receptors, have been described (Fig. 1). The chemokine family is divided into 4 groups (CC, CXC, CX3C, and C) based on the relative positions of their amino-terminal cysteins. CC-chemokines, of which there are more than 25, have adjacent cysteins and are represented by chemokines such as monocyte chemoattractant protein (MCP-1); regulated upon, normal T cell expressed and secreted (RANTES); and macrophage inflammatory protein (MIP-1α). CXC-chemokines, of which there are more than 15, have cysteins separated by a single amino acid, for which chemokine interleukin-8 (IL-8) is their prototype. CX3C-chemokines, of which there are more than 15, have cysteins separated by a single amino acid, for which chemokine interleukin-8 (IL-8) is their prototype. CX3C family, in which cysteins are separated by 3 amino acids, contains a single chemokine known as fractalkine. The C family, in which there is only 1 cystein, contains 2 chemokines, lymphotactin-α and lymphotactin-β.

Chemokines are produced by a variety of cell types of hematopoietic and non-hematopoietic origin, and their actions are mediated by a family of seven-transmembrane spanning, G-protein-coupled receptors (CCRs, CXCRs, CXCR1, and XCR1), which transduce intracellular signals following ligand binding.

Chemokines have multiple functions. In addition to promoting leukocyte chemotaxis, chemokines have been shown to be important in embryogenesis, angiogenesis, and initiation of cellular and humoral immune responses. Furthermore, specific chemokines have other proinflammatory properties, including stimulation of increased integrin expression and cell adhesion, calcium mobilization in T cells, increased T cell production of proteases leading to tissue infiltration, neutrophil degranulation, and monocyte superoxide production. These proinflammatory properties suggest that in vivo chemokines not only induce cellular recruitment but also amplify the level of tissue inflammation. The potential proinflammatory role of chemokines is further supported by the detection...
of many different chemokines, or their transcripts, at the sites of various inflammatory reactions.

Chemokines have been shown to play a pathogenic role in several inflammatory disorders, such as inflammatory bowel disease, bronchial asthma, rheumatoid arthritis, encephalomyelitis, and infection with human immunodeficiency virus. Their role in the pathogenesis of cardiovascular diseases will be the main focus of this review article.

Chemokines and Aging

The remodeling of the cytokine network is complex, with a shift from a type 1 (IFN-\(\gamma\) and IL-2) to a type 0 and type 2 (IL-4, IL-5, and IL-10) cytokine profile as age increases. Such remodeling also extends to a chemokine profile where a significant increase in chemokine production of MCP-1 and RANTES has been noted in centenarians compared to healthy young participants. In another study, MIP-1 and RANTES production was upregulated in participants between the ages of 86 and 95 years. Therefore, chemokines may have a compensatory role in mediating mechanisms that are called upon to promptly balance the age-related modification of immune response to maintain a healthy status in the elderly.
Chemokine Expression in Myocarditis

It is estimated that 10% to 20% of patients with clinically manifested myocarditis develop dilated cardiomyopathy. Most of our knowledge concerning immunopathology of acute myocarditis has been derived from the murine coxsackievirus B (CVB) myocarditis model. Cytotoxic T cells, neurohumoral factors, and free radicals play a significant role in the development of myocardial damage.

Investigators have analyzed cytokine expression in murine hearts with CVB3-induced myocarditis and have found high levels of TNF-β, IL-2, and Interferon-γ. The role of chemokines in the pathogenesis of CVB3 myocarditis has recently been explored. Cook et al. demonstrated in vivo the involvement of macrophage inflammatory protein (MIP-1α) in the inflammatory response in murine CVB3 myocarditis. Using knockout mice has shown that homozygous MIP-1α mutant (–/–) mice were resistant to CVB3-induced myocarditis. Furthermore, CVB3 infection has the potential to accelerate the production of MIP-2, a major macrophage and lymphocyte chemoattractant. Treatment with anti-MIP-2 monoclonal antibody prevents myocardial inflammatory and necrotic responses, resulting in reduced macrophage and T-cell accumulation.

These findings suggest that a blockade of chemokine expression may greatly limit myocarditis and the subsequent development of myocardial damage.

Enhanced production of monocyte chemoattractant protein-1 (MCP-1) in human myocarditis has recently been described by Fuse et al., who have shown that high plasma level of MCP-1 was associated with fatal outcome. Serum MCP-1 levels of 8 fatal cases (371.8 ± 145.2 pg/ml) were significantly (P = 0.0058) higher than those of 16 cases who survived (65.5 ± 12.8 pg/ml), suggesting that MCP-1 may play an important role in the pathogenesis of human acute myocarditis.

Chemokines and Congestive Heart Failure

Immunologic and inflammatory processes seem to be involved in the pathogenesis and progression of congestive heart failure (CHF). Proinflammatory cytokines, such as TNF-α, IL-1, and IL-6, have been shown to be capable of inducing myocardial dysfunction. Chronic low-grade inflammation with infiltrating leukocytes has been described in human chronic heart failure. CXC-chemokines may be important mediators in the persistent immune activation observed in CHF patients by activation of circulating neutrophils, T cells, and monocytes, and possibly by the recruitment of these cells into the failing myocardium. Damas et al. demonstrated elevated circulating plasma levels of 3 different CXC-chemokines, IL-8, growth-regulated oncogene (GRO)α, and epithelial neutrophil activating peptide (ENA-78) in patients with heart failure, both idiopathic dilated cardiomyopathy and ischemic cardiomyopathy. The highest levels were seen in patients with advanced disease, NYHA class IV. Interestingly, a significant inverse correlation was found between IL-8 and left ventricular ejection fraction (r = −0.67, P < 0.01) and cardiac index (r = −0.42, P < 0.01). In another study, enhanced gene expression of both CC (MIP-1α and MIP-1β) and CXC (IL-8) chemokines and their corresponding receptors (CCR1, CCR2, CCR5, CXCR1, CXCR2, and CX3CR1) was found in mononuclear blood cells from patients with heart failure. Gene expression of MIP-1α (r = −0.66, P < 0.01), MIP-1β (r = −0.52, P < 0.05), and their common receptor CCR1 (r = −0.64, P < 0.01) was inversely correlated with left ventricular ejection fraction. These findings support a role for chemokines in the pathogenesis of CHF.

Chemokine gene expression at the tissue level has also been explored, with the main findings of down-regulation of chemokine mRNA of MCP-1, IL-8, RANTES, and MIP-1α, and up-regulation of chemokine receptor mRNA of CCR1, CCR2, and CXCR1 in the failing myocardium. Chemokine expression dysregulation may represent one of several different maladaptive mechanisms responsible for progression of heart failure.

Chemokines may potentially modulate myocardial function both directly, through effects on cardiomyocytes, and indirectly, through effects on infiltrating leukocytes, fibroblasts, or endothelial cells within the myocardium. Mechanisms of chemokine action may include activation and production of reactive oxygen species, matrix metalloproteinases, and inflammatory cytokines.

Chemokines may also regulate other biologic processes.
processes, such as fibrosis and apoptosis, that are of importance to the pathogenesis of heart failure.\textsuperscript{52}

Chemokines in Myocardial Ischemia and Coronary Atherosclerosis

Animal experimentation have shown that ischemia-reperfusion injury is associated with generation of free radicals that activate nuclear factor-kB (NF-kB) and trigger the expression of various cytokines, such as IL-1\textbeta, IL-6, and TNF-\textalpha.\textsuperscript{53,57} Although reperfusion of ischemic myocardium is essential for myocyte survival, the restoration of blood flow to the ischemic myocardium is associated with an acute inflammatory response characterized by the activation and recruitment of neutrophils.\textsuperscript{60} Chemokines such as IL-8 and MCP-1 have been shown to be important mediators in neutrophil-mediated myocardial reperfusion injury in the rat model,\textsuperscript{59,60} and that neutralization of MCP-1 significantly reduces infarct size at 24 h after reperfusion.\textsuperscript{60} Ischemia-reperfusion injury has also been shown recently to induce other important chemokines such as lipopolysaccharide-induced CXC chemokine (LIX), cytokine-induced neutrophil chemoattractant (KC), and MIP-2. Furthermore, neutralization of LIX was associated with marked attenuation of myocardial neutrophil accumulation, again suggesting the importance of this chemokine in the injury process.\textsuperscript{41}

The role of chemokines in the pathogenesis of atherosclerosis has been investigated. Clinical studies have confirmed increased circulating levels of IL-8 and MCP-1 in patients with acute myocardial infarction, suggesting the importance of these chemokines as major contributors to the priming of neutrophils in these patients.\textsuperscript{52,53}

Enhanced expression of several chemokines, such as IL-8, MCP-1, interferon-g inducible protein 10 (IP-10), and eotaxin, has been described in human atherosclerotic lesions, mediating vascular medial thickening and rendering plaque instability.\textsuperscript{44,47} Furthermore, enhanced expression of chemokine receptors, CXCR2, CCR2, and CCR3, has also been reported in human atheromas.\textsuperscript{44,47} The importance of these chemokines in the pathogenesis of atherosclerosis has been well illustrated by knockout mice experiments where the lack of genes for MCP-1, IL-8, or CCR2 was associated with significant reduced progression of atherosclerosis.\textsuperscript{48,50}

Chemokines in the Cardiac Allograft

Chemokines and Acute Rejection

Acute allograft rejection and coronary vasculopathy are major causes of morbidity and mortality following transplantation. Acute rejection is mediated by the infiltration of alloantigen-specific T cells into the graft.\textsuperscript{51} Chemokine expression during rejection has been demonstrated in experimental and clinical studies.\textsuperscript{52} Fairchild noted 2 distinct patterns of chemokine mRNA expression in the rejecting cardiac allografts.\textsuperscript{53} The early phase, indicative of the inflammatory response induced by ischemia and trauma of the transplant surgery, is characterized by the expression of chemokines with macrophage and neutrophil chemoattractant properties such as macrophage chemoattractant JE, neutrophil chemoattractant KC, MCP-1, MIP-1\textalpha, and MIP-1\textbeta. The late phase is initiated when alloantigen-specific T cells infiltrate the graft and induce the production of proinflammatory cytokines such as IFN-\gamma, which in turn stimulates vascular endothelial cells to produce IP-10 and monokine induced by interferon-\gamma (Mig), which are potent chemoattractants for antigen-primed T cells.\textsuperscript{54-56} Upon graft infiltration, the primed T cells are activated to further induce cytokine production and cytolysis that mediate acute rejection and graft destruction.

In a recent study, IP-10 and Mig expression was demonstrated during rejection as early as day 2 posttransplant.\textsuperscript{56} This was accompanied by NK cell, CD4\textsuperscript{+}, and CD8\textsuperscript{+} T cell infiltration into the graft. Furthermore, antibody-mediated depletion of recipient CD8\textsuperscript{+} but not CD4\textsuperscript{+} T cells attenuated the expression of these chemokines, suggesting that their expression is mediated by the activities of CD8\textsuperscript{+} T cells.

Clinical studies have recently examined the role of chemokines in the pathogenesis of acute rejection. In an immunohistologic analysis of 65 endomyocardial biopsies from 30 patients, it has been shown that acute rejection was associated with expression of regulated upon activation, normal T cell expressed and secreted (RANTES) by infiltrating CD45RO\textsuperscript{+} memory T cells and macrophages.\textsuperscript{57} Re-
cently, Fairchild’s laboratory evaluated myocardial chemokine gene expression in heart transplant patients undergoing serial endomyocardial biopsies over 1 year. Acute rejection was associated with significant increased expression of IP-10, Mig, IFN-inducible T cell α-Chemoattractant (ITAC), and RANTES and their corresponding receptors, CXCR3 and CCR5, again confirming the results of earlier animal experiments.

The impact of donor brain death on host responsiveness to the cardiac allograft has recently been investigated by Wilhelm et al. who noticed an accelerated rate of acute rejection in hearts from brain-dead rats compared to hearts from living donor controls. This response was associated with an increased expression of cytokines (TNF-α, IL-1β) and chemokines (in particular, MCP-1), contributing to the up-regulation of the adhesion molecules (ICAM-1, VCAM-1) and causing leukocytes to infiltrate hearts from brain-dead donors earlier and in greater density than control donor hearts. MCP-1 also seems to be important in the pathogenesis of myocardial reperfusion injury by its ability to attract macrophages and monocytes via induction of ICAM-1 expression in myocytes and vascular smooth muscle cells. The increased chemokine expression in hearts from brain-dead donors provides another mechanism of graft injury besides the traditional known catecholamine-mediated injury seen following brain death.

Chemokine neutralization may be an attractive approach to attenuate the inflammatory response. Targeting different chemokine receptors such as CCR1, CCR2, CCR5, CX(3)CR1, and most notably CXCR3, has been found to be an effective strategy in inhibiting alloantigen-primed T-cell recruitment to the allograft and prolonging allograft survival. Furthermore, using specific monoclonal antibodies against IP-10 and KC has also been shown to prolong allograft survival.

Chemokines and Allograft Vasculopathy
Coronary vasculopathy is characterized by infiltration of CD4+ and CD8+ T lymphocytes, macrophages, and smooth muscle cell accumulation, resulting in diffuse concentric intimal thickening. In addition to leukocyte recruitment, chemokines facilitate tight binding of leukocytes to the endothelium after selectin-mediated rolling and enhance integrin expression and antigen-specific lymphocyte and monocyte activation. Yun et al. demonstrated a dual pattern of chemokine production in a murine model of allograft vasculopathy.

First, there is an early intragraft MIP-2 and MCP-1/JE production that paralleled neutrophil and macrophage infiltration, and this is likely related to ischemia-reperfusion phenomenon. Proinflammatory cytokines such as IL-1β and TNF-α, produced during ischemia-reperfusion injury, have been shown to induce MCP-1/JE and MIP-2 production in cardiac myocytes. A late chemokine profile is characterized by sustained lymphotactin, RANTES, and IP-10 expression that correlates with persistent macrophage and T lymphocyte infiltration and precedes intimal thickening and development of coronary vasculopathy. Similar findings were also noted by Fairchild et al. who observed persistent IP-10 and RANTES gene expression in the animal allograft vasculopathy model. In vitro studies have shown that IP-10 also stimulates the proliferation and migration of smooth muscle cells, which may suggest that chemokines may influence the development of vasculopathy through effects beyond leukocyte cell recruitment.

Cytomegalovirus (CMV) has been implicated in the pathogenesis of allograft vasculopathy, and its link to chemokine expression has recently been investigated. CMV has evolved various molecular mechanisms to both control host cell metabolism and evade immune surveillance. Among the viral gene products that are likely to be involved in these processes are homologues of cellular G protein-coupled receptors, MHC class I molecules, and chemokines. Cytomegalovirus infection of vascular cells induces expression of proinflammatory adhesion molecules by paracrine action of secreted IL-1beta. De La Melena et al. demonstrated an increased CC-chemokine (MCP-1, RANTES) expression in CMV-infected allografts that was associated with the development of accelerated vasculopathy.

Summary
In summary, chemokines play an important role in the pathogenesis of atherosclerosis, myocarditis, myocardial failure, ischemia-reperfusion injury, allograft acute rejection, and transplant vasculopathy.
Importantly, chemokine antagonism may represent a novel therapeutic strategy to alter the natural course of these different disease processes.

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