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REVIEW

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Chemokines, receptors, and their role in cardiovascular pathology

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Abstract A superfamily of leukocyte chemotactic proteins, known as chemokines, has been identified during the past decade. Chemokines selectively attract and activate different leukocyte subpopulations and are key mediators of a variety of patho-physiological states, including hematopoiesis, inflammation, infection, allergy, atherosclerosis, reperfusion injury, as well as malignant tumors. Chemokines bind and activate a number of specific or promiscuous, G-protein-coupled seven-transmembrane receptors. Some of these receptors are utilized by human immunodeficiency virus type 1 as essential fusion co-factors. Further understanding of the role of chemokines and their receptors in host defense will help develop means by which the beneficial versus detrimental effects of these molecules can be balanced.

Key words Chemokines · Receptors · Atherosclerosis · Reperfusion

Introduction

Leukocyte infiltration into inflamed or injured tissues requires a variety of cell-associated and soluble factors which mediate the communications between circulating leukocytes and vascular cells. Over the past decade, a superfamily of polypeptide leukocyte chemoattractants, known as chemokines, which comprise the largest subset of cytokines identified to date, have been demonstrated to selectively induce rapid endothelial cell adhesion and transmigration of leukocyte subpopulations [1–4]. Chemokines are characterized by their ability to induce directional migration and activation of leukocytes. They regulate leukocyte adhesion, angiogenesis, leukocyte trafficking and homing, and contribute to lymphopoiesis and hematopoiesis [1–4]. Chemokines are produced by a variety of cell types, including those of hematopoietic and non-hematopoietic origin, in response to antigens, polyclonal stimulants, cell irritants, as well as cytokines. A number of chemokines have been detected locally in the course of many disease states [1–5]. Chemokines bind and activate cell surface receptors that belong to the seven transmembrane, G-protein-coupled receptor (7TMR) superfamily [6]. Several chemokine receptors have been identified as fusion co-factors for human immunodeficiency virus type 1 (HIV-1) [7, 8]. In vivo studies using neutralizing antibodies, antagonists, or by deletion of chemokine and chemokine receptor genes have revealed that chemokines and their receptors play a pivotal role in host defense against microorganisms, including HIV-1 [2, 7, 8] and in inflammatory or immunological conditions such as ischemic reperfusion injury, adult respiratory distress syndrome, immune-complex-induced glomerulonephritis, atherosclerosis, autoimmune reactions, and malignant tumors [3, 5, 9]. As chemokine research is a rapidly expanding area, it is the purpose of this review to summarize recent progress in the field and to focus on the involvement of chemokines in cardiovascular diseases.

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Chemokine ligands

Four chemokine subfamilies (CXC, CC, C, and CX3C) are classified based on their primary amino acid sequences

Table 1 Human CXC, C, and CX3C chemokines and receptors

Chemokine	Receptor[a]	Cell source	Inducers	Target cells[b]
CXC				
IL-8	CXCR1, CXCR2	M, N, F, EC, K, NK, T, SMC, tumor cells	LPS, mitogen, particulates, microorganisms, IL-1, TNF	N, T, MC, EC, NK. B
GRO (α, β, γ)/MGSA	CXCR2, Possibly CXCR1	M, F, EC, melanoma cells	LPS, IL-1, TNF	N, MC, EC, F, fresh T
NAP-2	CXCR2, CXCR1	Platelets, F, EC	Platelet activation	N, EC
ENA-78	CXCR2, CXCR1	K, F, EC, SMC, epithelial cells	IL-1, LPS, TNF	N, EC
GCP-2	CXCR1, CXCR2	Osteosarcoma cells		N, EC
PF4	?	Platelets	Platelet activation	F, EC
IP-10	CXCR3	M, T, F, EC, K	IFN, TNF, LPS	T, NK, M, EC
MIG	CXCR3	M, hepatocytes	IFN-γ	T, EC
SDF-1	CXCR4	Bone marrow stromal cells	Constitutive	T, B, N, M
C				
Lymphotactin	?	T, NK		Thymocytes, T, DC, NK
CX3C				
Fractalkine/neurotactin	CX3CR1	EC, M, microglial cells	TNF, IL-1	T, M, N

B, B lymphocytes; EC, endothelial cells; F, fibroblasts; K, keratinocytes; M, monocytes; N, neutrophils; NK, natural killer cells; SMC, smooth muscle cells; T, T lymphocytes; IL-8, interleukin-8; IP-10, interferon-induced protein; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IFN, interferon; DC, dentritic cells

[a] CXCR4 is a fusion co-factor for T lymphocyte tropic human immunodeficiency virus-1 (HIV-1)

[b] The effect on EC includes induction of angiogenesis, presumably due to migration and proliferation of EC in response to CXC chemokines with ELR motif (IL-8, GRO, NAP-2, GCP-2, and ENA-78), or the inhibition of angiogenesis by CXC chemokines lacking ELR motif (PF4, IP-10, and MIG) [1, 3]

Table 2 Human CC chemokines and receptors

Chemokine	Receptor[a]	Main cell sources	Major inducers	Main target cells
MCP-1	CCR2b, CCR10	All cell types	LPS, mitogens, irritants, growth factors, Microorganisms, IL-1, TNF	M, T, SMC, NK, Bas, DC
MCP-2	CCR1, CCR2b, CCR5	Osteosarcoma, M, F	IFN, IL-1, virus, mitogens, LPS	M, T, NK, E, Eo, Bas, DC
MCP-3	CCR1, CCR2b, CCR3, CCR10	Osteosarcoma, M	IFN, mitogen, LPS	M, T, N, NK, Eo, Bas, DC
MCP-4	CCR2b, CCR3	M, Epi	Inflammatory stimuli	M, Bas, Eo
MCP-5	CCR2	M, SMC	Inflammatory stimuli	As MCP-1
ELC	CCR7	Lymphatic tissue	Inflammatory agents	M, T
Eotaxin	CCR3	EC, M, Epi, lung, Lym	Inflammatory/allergic agents	Eo
I-309	CCR8	M, Mas	Inflammatory agents	T, M
LARC/MIP-3α	CCR6	M, DC, liver/lung tissue	Inflammatory agents	M
MDC	CCR4	Mac, thymus	Constitutive	DC, M, NK
MIP-1α	CCR1, CCR3, CCR5	M, T, Mas, F	Mitogens, inflammatory agents	M, T, NK, DC, N, Eo, Stem cells
MIP-1β	CCR5	M, T, F	Inflammatory agents	M, T, NK, DC, Stem cells
RANTES	CCR1, CCR3, CCR5	T, EC, platelets	Inflammatory agents, mitogens	M, T, NK, DC, Eo, Bas
TARC (TECK)	CCR4	DC, EC	Inflammatory agents	M, T

Bas, basophils; MIP, macrophage inflammatory protein; Eo, eosinophils; Epi, epithelial cells; Lym, lymphocytes; Mas, mast cells; MCP, monocyte chemotactic protein

[a] CCR9 has been revealed in communication, but not published. CCR2b, CCR3, CCR5, CCR8 are fusion co-factors of various strains of HIV-1

[2, 3]. Their cell sources, inducers, target cells, as well as shared and unique receptors, are listed in Tables 1 and 2. CXC subfamily members have one amino acid (X) interrupting the first two of their four conserved cysteine residues. The genes of the CXC chemokines are clustered on human chromosome 4 (q12–21), whereas the SDF-1 gene is located on chromosome 10. CC chemokines have no intervening amino acid between the first two of their four (or in the case of I-309 six) cysteine residues, and their genes are located on chromosome 17 (q11–21), except for four newly identified members, the genes for which are located on chromosomes 2, 9, and 16. In both CXC and CC subfamilies, disulfide bonds are formed between the first and third, and between the second and fourth cysteines to form a stable tertiary structure with a molecular mass of 7–9 kilodaltons (kDa). Lymphotactin [10], the only mem-

ber of the C chemokine subfamily, is 16 kDa and its gene is located on chromosome 11. CX3C chemokine, known also as fractalkine or neurotactin, has three intervening amino acids between the N-terminal cysteines and its gene is on chromosome 16 [11, 12]. Fractalkine or neurotactin, with a molecular mass of 38 kDa, is larger than any other known chemokine. Its 18-amino acid hydrophobic sequence at the C-terminus appears to be an endothelial cell membrane docking motif, and a chemokine component of 80 amino acids can be cleaved from its 240-amino acid mucin-like stalk. However, fractalkine or neurotactin appears to function mostly in a cell-associated manner. Chemokines possess heparin-binding capacity at their C-terminal end, which enables them to bind to glycosaminoglycan and other negatively charged sugar moieties on cell surfaces and matrix glycoproteins [13]. This property results in the adsorption of chemokines onto the endothelial cell lining of the blood vessels, connective tissues, and cell matrices. Therefore, chemokines immobilized on surfaces may induce "haptotactic" migration of target cells [14].

Chemokine receptors

Four receptors for CXC chemokines (CXCR1–4) and 10 for CC chemokines (CCR1–10) have been identified and cloned (Tables 1, 2). Recently, the receptor for CX3C chemokine fractalkine/neurotactin has also been identified [15]. They belong to a subfamily of 7TMR [6], and show some homology to 7TMR for other proinflammatory chemoattractants such as platelet-activating factor (PAF), leukotriene$_4$, activated complement component 5 (C5a), and formyl-methionyl-leucyl-phenylalanine. Chemokine receptors are also structurally related to the receptors for a number of neurotransmitters [6].

A chemokine receptor consists of a hydrophobic seven transmembrane domain, an N-terminus outside the cell surface, three extracellular and three intracellular loops, and a C-terminus in the cytoplasmic compartment [6]. Chemokines exhibit high-affinity binding to the N-terminus of the receptor and a secondary site on one of the extracellular loops. The C-terminus of the receptors contains a number of serine and threonine residues which, upon phosphorylation, participate in signalling and receptor desensitization. One of the intracellular loops of the chemokine receptors couples with heterotrimeric G-protein, and ligand binding to the receptor initiates a cascade of signal transduction events. Chemokine-induced receptor signalling is often sensitive to inhibition by pertussis toxin, which uncouples selected G-proteins from the receptor [16, 17]. Activation of the receptors by chemokine ligands induces the exchange in the α subunit of the G-proteins from the GDP to GTP bound state, dissociating the α from the β and γG protein subunits. These subunits activate phospholipase (PL) Cβ1 and 2, followed by hydrolysis of phosphatidylinositol 4,5-biphosphate, which leads to the formation of inositol triphosphate (IP3) and diacylglycerol (DAG). By mobilizing intra- and/or extracellular calcium (Ca^{2+}), IP3 mediates the capacity of chemokines to induce Ca^{2+} flux in many cells types [16, 17]. DAG activates protein kinase (PK) C, which phosphorylates a number of effector molecules, such as mitogen-activated protein kinases (MAPKs). Chemokines also induce tyrosine phosphorylation in many cell types, involving the downstream MAPK cascade. Signals dependent on G-proteins result in actin polymerization, reconstitution of adhesion molecules and other cellular components leading to cell migration, while tyrosine phosphorylation and DAG/PLD activation promote superoxide production. The signals resulting in the cell migration response can be dissociated from Ca^{2+} and PKC-dependent signalling pathways [6, 16, 17].

There is a remarkable adaptation of chemokine receptors by microorganisms to their own advantage. The Duffy antigen, known as DARC, was identified as a 7TMR which binds several CXC and CC chemokines and is also used by *Plasmodium vivax* malaria parasites for invasion of human red blood cells [18]. DARC has been detected on postcapillary venule endothelial cells, Purkinje cells in the brain, and activated T lymphocytes, but it does not transduce chemokine signals. It is therefore considered as a "sink" for transportation and clearance of excessive chemokines [19]. A gene from human herpes virus 8 coding for chemokine receptor is detected in Kaposi's sarcoma of acquired immunodeficiency syndrome (AIDS) patients [20]. This receptor is highly homologous to receptors for the CXC chemokine interleukin-8 (IL-8) (CXCR1 and CXCR2) and constitutively transduces signals. Thus, it may promote tumor angiogenesis due to an enhanced vascularization [21]. The open reading frame of the cytomegalovirus (CMV) genome contains three 7TMR sequences, one of which, US28, codes for a functional receptor for multiple CC chemokines [6]. Over the past 2 years, chemokine receptors have been identified as major fusion co-receptors along with cellular CD4 for HIV-1 [2, 7, 8]. CXCR4, which is a receptor for the CXC chemokine SDF-1, mediates the entry of T lymphocyte tropic HIV-1. Whereas CCR5, whose natural ligands are macrophage inflammatory protein (MIP)-1α, MIP-1β, RANTES [2, 3, 6], and monocyte chemotactic protein (MCP)-2 [22], is a fusion co-factor for monocyte tropic HIV-1 viruses. Other chemokine receptors, such as CCR2, CCR3 [2, 7, 8], CCR8 [23], and CMV-US28 [24], have also been reported to mediate cellular invasion by certain HIV-1 strains . Dual-tropic HIV-1 strains may use both CXCR4-CCR5 [2, 7, 8] or STRLL33 on lymphocytes [25] for cell entry. In this context, chemokine ligands specific for these receptors are able to competitively inhibit HIV-1 entry and replication in $CD4^+$ cells, consequently selected chemokines constitute a group of potent natural antagonists to HIV-1 [22, 23, 26, 27].

The pleiotropism and a seeming redundancy of chemokines and their receptors are based on the capacity of a given chemokine to use multiple receptors and the capacity of many of the receptors to react to multiple chemokines. In addition, all leukocyte subsets express a multiplicity of chemokine receptors (Tables 1, 2). However, by using the gene-targeting approach, evidence is acumulating that each chemokine or receptor may have its unique

place in various diseases. Disruption of the CXC chemokine SDF1 gene resulted in severely reduced B lymphocyte progenitors and prenatal death of the mice [28]. Disruption of the gene encoding the CC chemokine eotaxin partially reduced antigen-induced tissue eosinophilia [29]. While deletion of the MIP-1α gene protected mice from Coxsackie virus-associated myocarditis [30], disruption of the gene for CCR1, one of the MIP-1α receptors, yielded mice resistant to pulmonary inflammation secondary to acute pancreatitis, possibly due to decreased production of tumor necrosis factor-α (TNFα) [31]. CCR1–/– mice also showed a disordered myeloid progenitor distribution, proliferation, and trafficking. Neutrophils in CCR1–/– mice failed to respond to MIP-1α in vitro and in vivo, accompanied by accelerated mortality when the mice were challenged with *Aspergillus fumigatus*, a fungus principally controlled by neutrophils [32]. On the other hand, the CCR1–/– mice exhibited an increased Th1 response to *Schistosoma mansoni* egg injection, with reduced granuloma formation in the lung [32]. Deletion of another CC chemokine receptor CCR2 which has at least five ligands (MCP-1 through MCP-5) resulted in failure of macrophages to accumulate in inflamed peritoneum and the CCR2–/– mice failed to clear infection by the intracellular bacteria, *Listeria monocytogenes* [33]. Human subjects with a homozygous mutation in HIV-1 co-receptor CCR5 showed remarkably prolonged resistance to HIV-1 infection, and AIDS patients with heterozygous CCR5 mutation exhibit longer survival [34, 35]. These observations suggest a fine-tuned chemokine and chemokine receptor network in the homeostasis in which one chemokine ligand or receptor plays a critical role.

Chemokines, atherosclerosis, and reperfusion injury

The role of chemokines in the cardiovascular system has been the focus of vigorous research. Vascular cells play an active role in hemostasis, inflammation, and immunity. Endothelial cells and smooth muscle cells (SMCs) produce and respond to a variety of cytokine mediators, such as IL-1 and TNF, which activate proinflammatory and prothrombotic functions of blood vessel cells [36, 37]. The actions of these inflammatory cytokines on vessel endothelial cells favor leukocyte extravasation by inducing expression of adhesion molecules and production of prostanoids, PAF, as well as chemokines, notably IL-8 and MCP-1 [38–42]. SMCs, as a major component of blood vessels (muscular arteries), are involved in vascular diseases such as atherosclerosis and vasculitis. Cells of the monocyte-macrophage lineage were identified in and around vessel walls affected by vasculitis. Monocyte extravasation represents an early event in atherosclerosis. Minimally modified low-density lipoproteins (MM-LDL), which are among the key elements involved in the formation of atherosclerosis [43], were able to stimulate endothelial cells of the large blood vessel to produce monocyte chemoattractant [44]. It was later shown to induce MCP-1 and IL-8 production by both human endothelial cells and SMCs [45]. Proinflammatory cytokines, such as IL-1 and TNF, also induce MCP-1 and IL-8 production by both endothelial cells and SMCs in vitro [38–42]. However, although the sequence in which proinflammatory cytokines and chemokines participate in the progress of atherosclerosis remains unclear, each type of mediator can be produced in response to the initial blood vessel wall injury, and both types are necessary in the development of an inflammatory/immunological process: the cytokines induce adhesion proteins on endothelial cells while chemokines induce complementary integrins on leukocytes [1, 3].

The expression and release of MCP-1 in macrophage-abundant arterial wall areas in human atherotic patients [1, 5] were detected by Northern blot analysis and in situ hybridization, as well as immunohistochemistry. MCP-1 was also found to be present in macrophage-derived foam cells in the lesions induced by a balloon catheter in rabbits fed a high-cholesterol diet. Although MCP-1 is not detected in sublesional SMCs and in normal arterial tissues [46], its production can be induced in SMCs and mesenchymal cells of the intima [47]. MCP-1 production was also demonstrated in SMCs of the medial layer of the arterial wall and in monocytes and SMC-like cells overlying intimal lesions in primates with hypercholesterolemia induced by a specific diet [48].

Co-culture of endothelial cells and SMCs from human arteries has been used to investigate the mechanisms of monocyte recruitment and formation of foam cells in arterial wall [49]. LDL significantly increased the expression of MCP-1 mRNA and the production of MCP-1 protein by co-cultured cells. A subsequent massive transmigration of monocytes into the subendothelial space was largely inhibitable by anti-MCP-1 antibody. The effect of LDL on blood vessel cells was blocked by high-density lipoprotein (HDL) and antioxidants [49].

The possible mechanisms of chemokine involvement in atherosclerosis are summarized in Fig. 1. LDL becomes trapped in the microenvironment of the extracellular matrix of the subendothelial space isolated from plasma and antioxidants. Reactive oxygen species or oxidized cellular lipids may be transferred to LDL, and this can then initiate the propagation of oxidated LDL. Since in the early stages of atherogenesis the subendothelial space is largely acellular and does not contain a significant number of monocyte-macrophages capable of releasing high levels of prooxidants, the resulting LDL is only minimally oxidized. This MM-LDL can then induce the production by overlying endothelium or underlying SMCs of MCP-1 with consequent expression of adhesion molecules for monocytes. These molecules in turn induce monocyte adhesion and transendothelial migration. The release of reactive oxygen intermediates and aldehydes further modifies the MM-LDL into a highly modified form which is recognized and taken up by macrophage scavengers, resulting in foam cell formation [43]. Furthermore, both MCP-1 and IL-8 are inducible in macrophage-foam cells in response to acetylated LDL loading, and IL-8 mRNA is detected in human coronary atheromas in the macrophage-rich area [50].

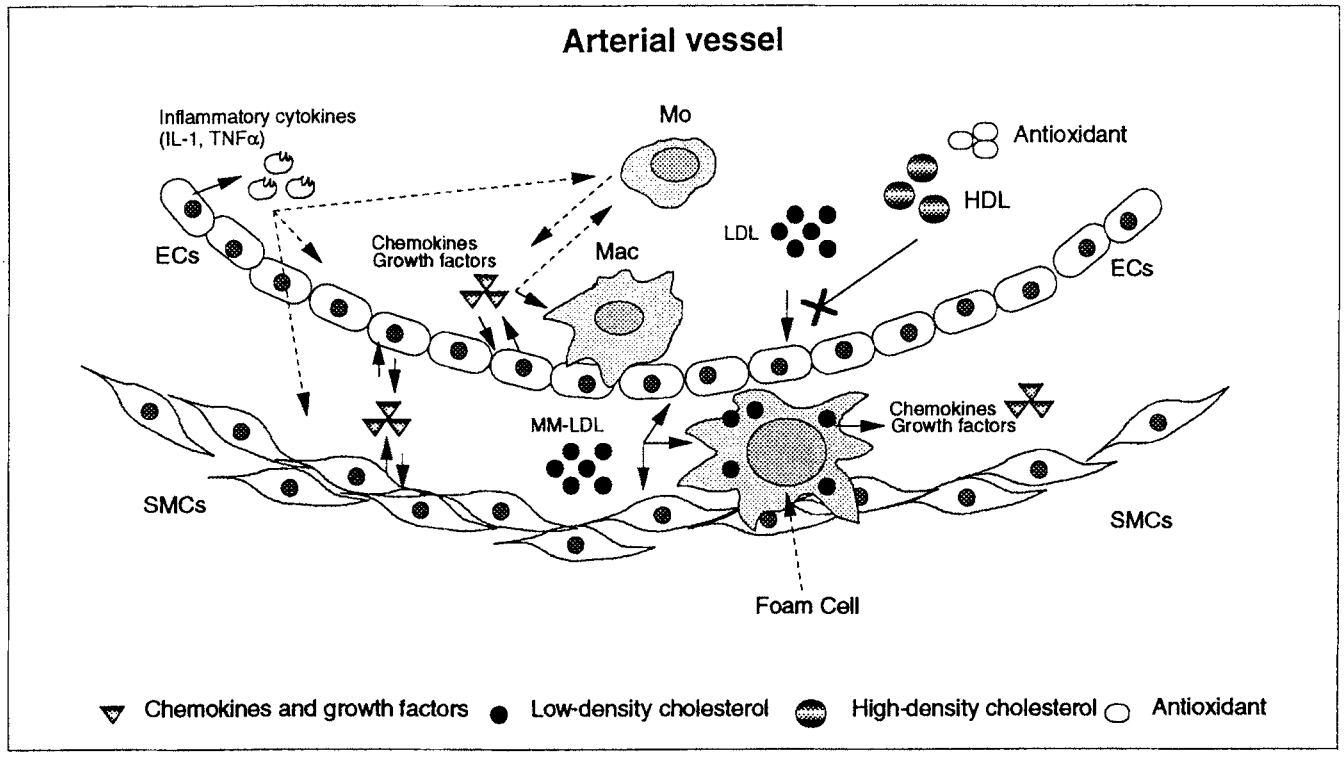

Fig. 1 Involvement of chemokines in atherosclerosis. Endothelial cells (*ECs*) and smooth muscle cell (*SMCs*) produce chemokines such as monocyte chemotactic protein-1 (*MCP-1*) and interleukin-8 (*IL-8*) upon vascular injury, stimulation by proinflammatory cytokines [IL-1, tumor necrosis factor-α (*TNF*α), etc.], or by oxidized low-density lipoprotein (*LDL*). MCP-1 (and other CC chemokines) induces transendothelial migration of blood monocytes (*Mo*)/macrophages (*Mac*), which subsequently become foam cells after ingesting LDL, and form fatty streaks. Foam cells also secrete chemokines in response to cholesterol loading. Chemokines and growth factors produced by ECs, SMCs, and foam cells induce proliferation of vessel cells and contribute to the atherosclerotic formation. High-density lipoprotein (*HDL*) and antioxidants are antagonists of the proinflammatory effect of LDL

However, in the presence of HDL or antioxidants in sufficient concentration, the formation of biologically active MM-LDL and the resultant inflammatory reaction can be prevented. Other studies have demonstrated high levels of mRNA for CC chemokines MIP-1α, MIP-1β, and RANTES [5] in carotid plaques, atherosclerotic lesions, and in atherosclerotic allogeneic heart transplant. In these instances, the CC chemokine mRNAs were co-expressed and could only be detected in the band of macrophages migrating through the neointima. The differential expression of CC chemokines by different cell types in arteriosclerosis suggests that chemokines may help create a network of signals at specific sites of the microenvironment to modulate the inflammation.

The chemokines have pleiotropic effects. MCP-1, in addition to its monocyte chemoattractant effects, has been reported to stimulate SMC proliferation [51]. The CXC chemokine IL-8, in addition to its neutrophil chemotactic properties, also has mitogenic and chemotactic activity on endothelial cells [52]. More recently, the CXC chemokine IP-10 (interferon-induced protein), which is chemotactic for T cells and monocytes, has been detected in human carotid arteries after balloon angioplasty. IP-10 can induce potent chemotactic migration and proliferation of SMCs [53].

Transplantation-associated accelerated atheroslerosis reduces the duration of survival and causes malfunction of the transplanted organs. Although the mechanism of its pathogenesis is not well understood, it is likely that an alloimmune response occurs leading to infiltration of the vessel wall by monocytes and T lymphocytes and consequent SMC proliferation and extracellular matrix deposition. Coronary arteries from patients undergoing re-transplantation owing to accelerated atherosclerosis were examined for expression of another CC chemokine RANTES, a potent chemotactic agent for monocytes and T cells, by in situ hybridization and immunohistochemistry [54]. RANTES mRNA and protein were detected in infiltrating lymphcytes and macrophages as well as in myofibroblasts and endothelial cells of arteries undergoing accelerated atherosclerosis, but not in normal coronary arteries. These studies showed that RANTES could also be a pivotal mediator of the cellular infiltrate seen in atherosclerotic allografts.

Chemokines contribute to an inflammatory cascade in reperfusion injury that is characterized by leukocyte invasion/activation and tissue destruction [55]. Cerebral reperfusion occurs after a hypoxic episode caused by vascular occlusion; the subsequent increased production of IL-1 and TNF promotes leukocyte adhesion to endothelial cells, possibly in part by inducing chemokines such as IL-8, which is known to attract neutrophil infiltration into brain

parenchyma [56]. This may account for the dramatic reduction in edema of the central nervous system and the size of the infarct by anti-IL-8 antibodies following carotid artery occlusion by balloon angioplasty [57]. Kim [58] investigated the expression of chemokines in ischemic rat brain following middle cerebral artery occlusion. MCP-1 immunoreactivity was diffusely expressed in the ischemic area and was most intense at 48 h after blood vessel occlusion. The major source of MCP-1 in the ischemic brain is endothelial cells and macrophages. Another monocyte chemotactic CC chemokine MIP-1α is concomitantly expressed in activated astrocytes. A crucial role of IL-8 in the development of lung reperfusion injury was demonstrated by the remarkably curative effect of anti-IL-8 antibodies [9, 57]. The anti-IL-8 antibodies, by blocking IL-8-induced adhesion and degranulation of neutrophils in injured pulmonary tissues, prevents the severe damage due to infiltrating neutrophils in reperfused hypoxic tissues. Although the source of chemokines in ischemic injury was believed to be mainly endothelial cells in response to hypoxia, cardiac myocytes have also been found to produce CC and CXC chemokines following ischemic stress [59], and thus may actively recruit leukocytes participating in the development and repair of reperfusion injury of myocardium.

Conclusions

Chemokines and their receptors have been demonstrated to be key mediators of a number of infectious, inflammatory, and immunological diseases. Chemokines and their receptors provide a unique opportunity to use bioassay screening approaches to identify antagonistic agents to counter the pathological effects of chemokines. Anti-chemokine antibodies, anti-IL-8 in particular, can reverse the acute consequence of reperfusion injury. Peptide analogues of chemokines, such as those of MCP-1 and RANTES, compete with native ligands for binding sites without inducing cell activation. These analogues were effective in inhibiting MCP-1-mediated mouse arthritis [60] and in blocking chemokine receptor-mediated HIV-1 fusion [26]. Human subjects with homozygous CCR5 mutation showed remarkably prolonged resistance to HIV-1 infection and AIDS patients with heterozygous CCR5 mutation exhibit longer survival [7, 8, 34, 35]. Furthermore, LDL and antioxidants have been shown to block the capacity of LDL to induce chemokines in vascular cells, thus preventing the inflammatory response of endothelial cells and SMCs to LDL [49]. These results indicate that while chemokines are important molecules in host defense, inhibition of excessive chemokine production or chemokine interaction with their receptors can effectively interrupt the development of a number of pathological states. This encourages a more-extensive in vivo study of the chemokine antagonists in cardiovascular diseases.

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