Inflamm. res. 53 (2004) 585–595
1023-3830/04/110585-11
DOI 10.1007/s00011-004-1298-5

Inflammation Research

Reviews

Chemokines in the ischemic myocardium: from inflammation to fibrosis

N. G. Frangogiannis

Section of Cardiovascular Sciences, The Methodist Hospital and the DeBakey Heart Center, One Baylor Plaza M/S F-602, Houston TX 77030, USA, Fax: ++713 796 0015, e-mail: ngf@bcm.tmc.edu

Received 2 April 2004; accepted by R. Pettipher 20 May 2004

Abstract. Myocardial infarction is associated with an inflammatory response leading to leukocyte recruitment, healing and formation of a scar. Members of the chemokine superfamily are rapidly induced in the infarcted myocardium and may critically regulate the post-infarction inflammatory response. CXCL8/Interleukin (IL)-8 is upregulated in the infarcted area and may induce neutrophil infiltration. In addition, mononuclear cell chemoattractants, such as the CC chemokines CCL2/Monocyte Chemoattractant Protein (MCP)-1, CCL3/Macrophage Inflammatory Protein (MIP)-1α, and CCL4/MIP-1β are expressed in the ischemic area, and may regulate monocyte and lymphocyte recruitment. However, chemokines may have additional effects on healing infarcts beyond their leukotactic properties. The CXC chemokine CXCL10/Interferon-γ inducible Protein (IP)-10, a potent angiostatic factor with antifibrotic properties, is induced in the infarct and may prevent premature angiogenesis and fibrous tissue deposition, until the infarct is debrided and provisional matrix necessary to support granulation tissue ingrowth is formed. Chemokine induction in the infarct is transient, suggesting that inhibitory mediators (such as transforming growth Factor (TGF)-β) may be activated suppressing chemokine synthesis and leading to resolution of inflammation and transition to fibrosis. Brief repetitive ischemia in mice also results in chemokine upregulation followed by suppression of chemokine synthesis and interstitial fibrosis, in the absence of myocardial infarction. Chemokine expression may play a role in the pathogenesis of non-infarctive ischemic cardiomyopathy, where early ischemia-induced chemokine expression may be followed by activation of inhibitory mediators that suppress inflammation, but induce fibrosis.

Key words: Inflammation – Myocardial ischemia – Chemokines – Leukocyte – Endothelium

Introduction

The chemokines [1–7] comprise a superfamily of small highly basic proteins with molecular weights in the range of 8–14 kDa and a strikingly similar tertiary structure [8]. Most chemokines contain at least four cysteines that form two disulfide bonds, one between the first and the third and one between the second and the fourth cysteine. Chemokines are subdivided into CC, CXC, or CX3C families based on the number of amino acids between the first two cysteines (Tables 1, 2). 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. Chemokines bind to heptahelical G protein-coupled receptors. Most receptors recognize more than one chemokine and certain chemokines may bind to several receptors.

Chemokines play a critical role in basal and inflammatory leukocyte locomotion and trafficking [9, 10] and their principal targets are bone marrow-derived cells. Most chemokines are secreted and in order to induce a chemotactic response in vivo they must be immobilized on cell or extracellular matrix surfaces through interactions with glycosaminoglycans [11]. In addition to effects on cell locomotion, certain chemokines are capable of eliciting a variety of other responses affecting leukocyte adhesion [12], activation and degranulation, mitogenesis, and apoptosis. It has been recently recognized that chemokines have a wide range of effects on many different cell types beyond the immune system, including endothelial cells (resulting in angiogenic or angiostatic effects) [13], smooth muscle cells, neurons and epithelial cells.

Table 1. Properties of the CXC, C and CX3C chemokines.

Systematic name	Human common name	Mouse common name	Expression	Receptors bound
CXC chemokine family				
CXCL1	GROα	GRO/MIP-2/KC?	Inducible	CXCR2 > CXCR1
CXCL2	GROβ	GRO/MIP-2/KC?	Inducible	CXCR2
CXCL3	GROγ	GRO/MIP-2/KC?	Inducible	CXCR2
CXCL4	PF4	PF4	Inducible	Unknown
CXCL5	ENA-78	GCP-2/LIX?	Inducible	CXCR2
CXCL6	GCP-2	GCP-2/LIX?	Inducible	CXCR1, CXCR2
CXCL7	NAP-2	Unknown	Inducible	CXCR2
CXCL8	IL-8	Unknown	Inducible	CXCR1, CXCR2
CXCL9	Mig	Mig	Inducible	CXCR3
CXCL10	IP-10	IP-10/CRG-2	Inducible	CXCR3
CXCL11	I-TAC	I-TAC	Inducible	CXCR3
CXCL12	SDF-1α/β	SDF-1/PBSF	Constitutive	CXCR4
CXCL13	BCA-1	BLC	Constitutive	CXCR5
CXCL14	BRAK/bolekine	BRAK		Unknown
(CXCL15)	Unknown	Lungkine		Unknown
CXCL16	CXCL16	CXCL16		CXCR6
C chemokines				
XCL1	Lymphotactin/ATAC/ SCM-1α	Lymphotactin		XCR1
XCL2	SCM-1β	Unknown		XCR1
CX3C chemokine				
CX3CL1	Fractalkine	Neurotactin/ ABCD-3	Both	CX3CR1

Table 2. Properties of the CC chemokines.

Systematic name	Human common name	Mouse common name	Expression	Receptors bound
CC chemokine family				
CCL1	I-309	TCA-3/P500	Inducible	CCR8
CCL2	MCP-1/MCAF	JE?	Inducible	CCR2
CCL3	MIP-1α	MIP-1α	Inducible	CCR1, CCR5
CCL3L1	LD78β	Unknown	Inducible	CCR1, CCR5
CCL4	MIP-1β	MIP-1β	Inducible	CCR5
CCL5	RANTES	RANTES	Inducible	CCR1, CCR3, CCR5
(CCL6)	Unknown	C10/MRP-1		Unknown
CCL7	MCP-3	MARC?	Inducible	CCR1, CCR2, CCR3
CCL8	MCP-2	MCP-2?	Inducible	CCR3, CCR5
CCL9	Unknown	MRP-2, MIP-1γ		
CCL10	Unknown	CCF18		
CCL11	eotaxin	eotaxin	Inducible	CCR3
(CCL12)	Unknown	MCP-5	Inducible	CCR2
CCL13	MCP-4	Unknown	Inducible	CCR2, CCR3
CCL14	HCC-1	Unknown		CCR1, CCR5
CCL15	HCC-2/MIP-1δ	Unknown		CCR1, CCR3
CCL16	HCC-4/LCC-1	Unknown		CCR1, CCR2
CCL17	TARC	TARC/ABCD-2	Inducible	CCR4
CCL18	DC-CK1/PARC	Unknown	Constitutive	Unknown
CCL19	MIP-3β/ELC-exodus-3	MIP-3β/ELC-exodus-3	Constitutive	CCR7
CCL20	MIP-3α/LARC/exodus-1	MIP-3α/LARC/exodus-1	Constitutive	CCR6
CCL21	6Ckine/SLC/exodus-2	6Ckine/SLC/exodus-2	Constitutive	CCR7
CCL22	MDC/STCP-1	ABCD-1	Both	CCR4
CCL23	MPIF-1/CKβ8	Unknown		CCR1
CCL24	Eotaxin-2/MPIF-2	MPIF-2	Inducible	CCR3
CCL25	TECK	TECK	Constitutive	CCR9
CCL26	Eotaxin-3	Unknown	Inducible	CCR3
CCL27	CTACK/ILC	ALP/CTACK	Constitutive	CCR10
CCL28	MEC	Unknown		CCR3/CCR10

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, and inducible chemokines which are strongly upregulated by inflammatory or immune stimuli, actively participating in the inflammatory reactions by inducing leukocyte recruitment [9, 14, 15]. Although this approach is oversimplified, it offers valuable insight into the role of certain chemokines in pathological states. A wide variety of stimuli can upregulate inducible chemokines, leading to a rapid, marked increase in their local concentration followed by leukocyte infiltration and an inflammatory response. Many cell types are capable of producing chemokines under appropriate conditions. Usually the same cell produces many chemokines concomitantly in response to the same stimulus (polyspeirism). Polyspeirism is particularly striking in endothelial cells and mononuclear phagocytes, which express many CC and CXC chemokines upon stimulation with pro-inflammatory cytokines or lipopolysaccharide.

The role of chemokines in cardiovascular disease

Expression of chemokines is found in a wide variety of disease processes, associated with tissue injury and leukocyte recruitment [9]. Involvement of chemokines in the pathobiology of conditions, such as multiple sclerosis, HIV disease, asthma, rheumatoid arthritis and neoplasia, has been inferred by animal model experiments and supported by correlative data in humans. Recent studies indicated a potential role for the chemokines in the pathogenesis of cardiovascular diseases, in particular atherosclerosis [16, 17] and cardiac allograft rejection [18, 19].

MCP-1 [20], IL-8 [21], IP-10 [22], Stromal Cell-Derived Factor (SDF)-1 [23], I-309 [24] and fractalkine [25] have all been identified in human atherosclerotic plaques. MCP-1/CCR2 interactions appear to have a central role in the pathogenesis of atherosclerosis: MCP-1 deficient animals have significantly less arterial lipid deposition in hypercholesterolemia models [26] and CCR2 deficiency has a similarly protective effect within an apoE deficiency model [27]. Furthermore, a decrease in atherosclerotic lesion formation was observed in mice deficient for the fractalkine receptor CX3CR1, suggesting a key role for this chemokine in atherogenesis [28]. Both CC and CXC chemokines have been implicated in the pathogenesis of cardiac allograft rejection and graft arteriopathy [29, 30].

Myocardial infarction is associated with an intense inflammatory response, that ultimately leads to healing and formation of a scar. Recent studies have demonstrated chemokine induction in the ischemic myocardium [31–34] and suggested involvement of these molecules in ischemic injury and repair, and in the pathogenesis of ischemic cardiomyopathy [35]. The current review will discuss the regulation and potential role of the chemokines in myocardial infarction and in non-infarctive ischemic cardiomyopathy. Understanding the function of chemokines in myocardial ischemia may lead to the development of specific therapeutic strategies aimed at optimizing cardiac repair.

Initiation of the inflammatory cascade in myocardial ischemia and reperfusion

Myocardial cell necrosis results in the release of subcellular membrane constituents, rich in mitochondria, which are capable of triggering the early acting components (C1, C4, C2 and C3) of the complement cascade [36]. By binding C1 and supplying sites for the assembly of later acting complement components, these subcellular fragments provide the means to disseminate the complement-mediated inflammatory response to ischemic injury. Generation of reactive oxygen intermediates may also be crucial for the initiation of the inflammatory response in the injured myocardium. They have the potential to directly injure cardiac myocytes and vascular cells and may be involved in triggering inflammatory cascades through the induction of cytokines and chemokines [37], and stimulation of leukocyte chemotaxis [38].

Complement activation and free radical generation appear to be important factors in triggering the cytokine cascade in the infarcted myocardium. A critical element in the regulation of cytokines and adhesion molecules in the ischemic myocardium involves the complex formed by Nuclear Factor (NF)-κB and IκB. NF-κB is activated by a vast number of agents, including cytokines (such as tumor necrosis factor (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, cell adhesion and growth control [39]. Studies from our laboratory [40] indicated a role for TNF-α in initiating the cytokine cascade ultimately responsible for intercellular adhesion molecule (ICAM)-1 induction in the reperfused canine myocardium.

Chemokine expression in experimental models of myocardial infarction

Chemokine upregulation is a prominent feature of the post-infarction inflammatory response in several mammalian species [41, 42] (Table 3). The CXC chemokines IL-8 and IP-10 and the CC chemokine MCP-1 appear to be consistently upregulated in various models of experimental myocardial infarction [41] and may play an important role in regulating leukocyte trafficking, wound angiogenesis and repair. The mechanisms responsible for chemokine upregulation in the ischemic heart have not been elucidated, however the factors implicated in initiating the inflammatory response (such as free radical generation, NF-κB activation, TNF-α release, and complement activation) are likely to stimulate, directly or indirectly, chemokine synthesis in the injured myocardium. Evidence suggests that chemokine induction in models of brief myocardial ischemia is mediated mainly by reactive oxygen intermediates [34, 43]. However, in myocardial infarcts cellular necrosis may trigger additional chemokine-inducing pathways and the relative contribution of free radical generation remains unclear. 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 [44]. Kilgore and co-workers [45] reported an attenuated IL-8 response accompanied by decreased neutrophil infiltration in C6-deficient rabbits, suggesting that the cytolytic membrane

Table 3. Chemokine expression in experimental models of myocardial ischemia and reperfusion.

Chemokine	Model	Reference	Presumed role	Cellular localization
CXCL8/IL-8	Dog/infarction	32	Neutrophil infiltration	Inflammatory cells, endothelium
CXCL8/IL-8	Rabbit/infarction	51	Neutrophil infiltration	Inflammatory leukocytes
CXCL1/GRO-α/KC	Rat/infarction	55	Neutrophil infiltration	Inflammatory leukocytes
MIP-2	Rat/infarction	55	Neutrophil infiltration	Inflammatory leukocytes
LIX	Rat/infarction	55	Neutrophil infiltration	Cardiomyocytes
CXCL10/IP-10	Dog/infarction	33	Angiostatic effect	Microvascular endothelium
SDF-1α	Rat/infarction	69		
MCP-1	Dog/infarction	31, 62	Mononuclear cell recruitment	Inflammatory leukocytes, endothelium
MCP-1	Rat/infarction	80, 81	Mononuclear cell recruitment	Macrophages
MCP-1/JE	Mouse/infarction	82	Myocyte survival	
MCP-1, MIP-1α, MIP-1β, MIP-2, IP-10	Mouse/infarction	90	Leukocyte infiltration	
MCP-1	Dog/brief (15 min) ischemia	34	Angiogenesis, Fibrosis	Microvascular endothelium
MIP-1α, MIP-1β, MIP-2	Mouse/brief (15 min) ischemia	43	Angiogenesis, Fibrosis	Microvascular endothelium
MCP-1, MIP-1α, MIP-1β	Mouse/brief (15 min) repetitive ischemia	121	Inflammation, Interstitial fibrosis	

attack complex plays an important role in regulating expression of the chemokine in the infarct. In addition, the rapid breakdown of extracellular matrix in injured tissues may result in accumulation of hyaluronan fragments, which are capable of inducing chemokine synthesis in macrophages [46] and endothelial cells [47].

Expression of CXC chemokines in myocardial infarcts

The prototypic CXC chemokine IL-8/CXCL8 was purified as a monocyte-derived factor that attracts neutrophils, but not monocytes, in Boyden chamber assays [2]. Several other CXC chemokines are also potent neutrophil chemoattractants and structure/activity analyses show that this property depends on the presence of the ELR (glutamate-leucine-arginine) motif, between the N-terminus and the first cysteine [8, 48]. IL-8 is a critical regulator of neutrophil influx and activation in inflammatory processes [49], however it also exerts potent angiogenic effects [50], and may play a role in wound healing and repair.

Interleukin (IL)-8 upregulation has been documented in canine [32] and rabbit [51] models of experimental myocardial infarction. In a canine model, 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 [32]. Recombinant canine IL-8 markedly increased adhesion of neutrophils to isolated canine cardiac myocytes [32], suggesting a potential role in neutrophil-mediated myocardial injury. The exact role of IL-8 in myocardial infarction remains unclear: a recent study suggested that IL-8 neutralization significantly reduces the degree of necrosis in a rabbit model of myocardial ischemia-reperfusion injury without affecting neutrophil infiltration [52]. Unfortunately, elucidating the role of IL-8 in myocardial infarcts using knockout and transgenic animals is hampered by the absence of an IL-8 homolog in the mouse.

Much less is known about the potential expression and role of other ELR-containing CXC chemokines in myocardial infarcts. Growth related 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 [53]. Independently, its murine homolog was cloned in a differential screening experiment as the platelet-derived growth factor (PDGF)-inducible KC gene [54]. GRO-α/KC, a potent neutrophil chemoattractant, is induced in a rat model of experimental myocardial infarction [55], however its role in regulating the post-infarction inflammatory response remains unclear. GRO-β/CXCL2 and GRO-γ/CXCL3 are closely related proteins that are also potent neutrophil chemoattractants; their expression in myocardial infarcts has not yet been studied. Epithelial Neutrophil Activating protein (ENA-78/CXCL5) is another ELR-containing CXC chemokine that exhibits similarities with the GROs. ENA-78 expression is induced in hepatic ischemia and reperfusion [56], however its function in myocardial infarction remains unknown. Deficiency of CXCR2, the main receptor for the ELR-containing CXC chemokines, resulted in significantly decreased inflammatory leukocyte recruitment in murine infarcts, suggesting a crucial role for these chemokines in inflammatory cell infiltration [57]. However, 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.

In contrast with ELR-containing chemokines, the CXC chemokines lacking the ELR motif, (such as platelet factor 4 (PF4/CXCL4), IP-10/CXCL10, and monokine induced by γ-interferon (MIG/CXCL9)), 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 either ELR-CXC chemokines or the unrelated angiogenic factor, basic fibroblast growth factor (bFGF) [13, 58]. In addition, IP-10 may have direct inhibitory effects on fibroblast migration [59], serving as an antifibrotic agent. A recent study from our laboratory demonstrated a marked transient upregulation of the angiostatic CXC chemokine IP-10 in reperfused canine myocardial infarcts [33]. IP-10 mRNA expression is downregulated following 24 h of reperfusion,

whereas IL-8 message levels remain high. IP-10 mRNA and protein was localized in the microvascular endothelium of ischemic myocardial segments [33]. In vitro experiments demonstrated that TNF-α, which is released early after myocardial ischemia [40] markedly upregulates IP-10 expression in canine venous endothelial cells [33, 60]. In order to investigate the mechanisms of IP-10 downregulation after 24 h of reperfusion, we studied the effects of IL-10 and TGF-β, both present in the ischemic myocardium [61, 62] 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. The exact role of IP-10 upregulation in the infarcted myocardium remains unclear. 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.

SDF-1 is a CXC chemokine with a critical role in cardiovascular development [63] and angiogenesis [64, 65]. In addition, SDF-1 induces chemotaxis of CD34+ progenitors [66] and primitive hematopoietic cells [67] and controls many aspects of stem cell function [68]. SDF-1α induction was recently reported in a rat model of non-reperfused myocardial infarction [69], however the role of this chemokine in regulating the post-infarction inflammatory response is unknown. Recent experiments identified bone marrow-derived stem cells in the infarcted myocardium [70, 71] suggesting that they may participate in cardiac repair. Although the mechanisms for stem cell homing in the ischemic myocardium remain unclear, SDF-1 may be an important factor regulating their recruitment, maturation and function in the infarct [72].

Expression of CC chemokines in myocardial infarction

CC chemokines are functionally diverse and their names more often reflect historical accidents of their cloning or isolation than their predominant functions [2]. 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 [73, 74]. Its expression and functional significance have been documented in a wide variety of disease processes, such as atherosclerosis [26, 75], multiple sclerosis [76], rheumatoid arthritis [77], stroke [78], and nephritis [79]. MCP-1 upregulation has been demonstrated in a canine [31], a rat [80, 81] and a murine model [82] of experimental myocardial infarction. In the canine model, induction of MCP-1 mRNA occurred only in ischemic segments within the first h of reperfusion, peaked at 3 h, and persisted throughout the first 2 days of reperfusion. In the absence of reperfusion, MCP-1 induction was significantly lower [31]. MCP-1 was localized by immunostaining on infiltrating cells and venular (but not arterial) endothelium by 3 h. Additional experiments suggested that MCP-1 may be a major factor responsible for mononuclear cell recruitment into the ischemic myocardium during the first five h of reperfusion [62]. In a rat model of experimental myocardial infarction, administration of a neutralizing antibody to MCP-1 significantly reduced infarct size decreasing adhesion molecule expression and macrophage infiltration [80]. However, MCP-1 may have important effects on infarct healing unrelated to its leukotactic actions, and mediated through its direct angiogenic effects on the vascular endothelium [83], or by direct modulation of fibroblast phenotype and activity [84]. Other studies suggested effects of MCP-1 on cardiomyocytes: in vitro experiments suggested that MCP-1 may promote the adhesion of neutrophils to myocytes via ICAM-1 expression [85]. In contrast, a recent study indicated that JE/MCP-1 markedly decreased hypoxia-induced cell death in cultured murine cardiac myocytes suggesting an unanticipated MCP-1-dependent cardiomyocyte survival mechanism [82]. MCP-1 may exert diverse effects on different cell types involved in the post-infarction inflammatory response; its exact role in myocardial injury and repair remains to be elucidated. Anti-MCP-1 gene therapy attenuated left ventricular dilatation in a murine model of experimental infarction, suggesting an important role for MCP-1 in post-infarction remodeling [86], however the specific mechanisms responsible for this effect remain unclear.

MIP-1α and MIP-1β were purified from lipopolysaccharide (LPS)-treated monocytic cell lines [87, 88] and are mononuclear cell chemoattractants, although less efficient than MCP-1 [89]. A robust induction of MIP-1α and MIP-1β is noted in murine infarcts [90], and MIP-1α levels are elevated in patients with myocardial infarction [42], however the importance of these chemokines in myocardial injury and repair has not been investigated. 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 lymphocytes [91]. RANTES, an important chemoattractant for monocytes, eosinophils, and specific subsets of T-cells [92] was found in the serum from patients with acute myocardial infarction [42], however information on its local expression in healing infarcts is lacking.

Role of chemokines in regulating specific cellular responses in healing infarcts (Fig. 1)

The role of chemokines in regulating neutrophil recruitment in the infarct

CXCR2–/– mice have decreased leukocyte infiltration in the infarct [57], suggesting direct involvement of CXC chemokines in recruitment of inflammatory cells. At the early stages of myocardial infarction IL-8 may be important in regulating neutrophil recruitment and activation. One of the earliest sequelae of reperfusion involves neutrophil trapping in the microvasculature. Engler and coworkers [93] demonstrated that entrapment of leukocytes in the microcirculation precedes their role in the inflammatory reaction. Neutrophils are large and stiff cells and may adhere to capillary endothelium preventing reperfusion of capillaries following coronary ischemia. The mechanism by which neutrophil trapping occurs in the microvessels is likely to be multifactorial. Chemotactic factors, such as IL-8, rapidly induce neutrophils to change shape and to become less deformable [94]. Neutrophils also release a variety of autacoids, such as thromboxane B2 which induce vasocon-

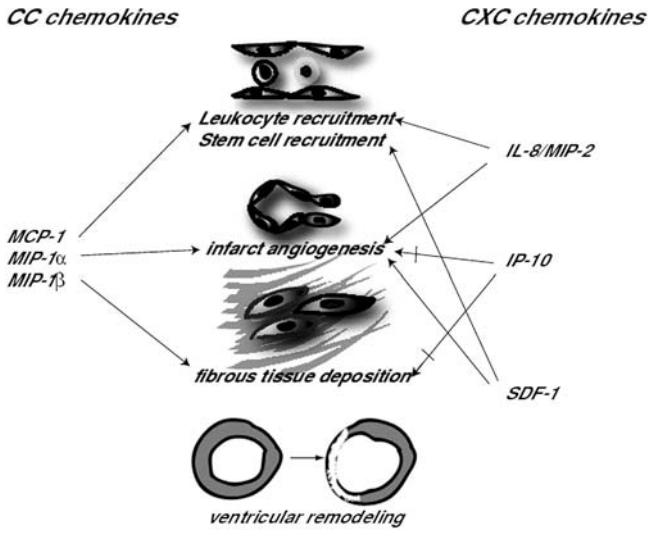

Fig. 1. Effects of chemokines on healing myocardial infarcts. Studies using experimental models of myocardial infarction have demonstrated that the CC chemokines MCP-1, MIP-1α and MIP-1β and the CXC chemokines IL-8, IP-10 and SDF-1 are induced in the infarcted myocardium. Chemokines may be crucial for recruitment of hematopoietic cells in the injured areas, however they may also modulate phenotype and gene expression in non-blood derived cells. Infarct angiogenesis and fibrous tissue deposition may be directly affected through MCP-1, IL-8 and IP-10 mediated mechanisms. IP-10, a potent angiostatic factor with anti-fibrotic properties may have a unique role in infarct healing delaying premature angiogenesis and fibrosis until the wound is debrided, and a provisional matrix necessary to support granulation tissue ingrowth is formed. Chemokine-mediated effects on specific cellular responses in the healing myocardium may modulate post-infarction ventricular remodeling.

striction and platelet aggregation and leukotriene B4 which induces neutrophil activation. Neutrophil interaction with endothelial cells via specific adhesion molecules results in their margination and adhesion to the endothelium.

There is increasing evidence that leukocyte-endothelial interactions are regulated by a cascade of molecular steps that correspond to the morphological changes that accompany adhesion. This adhesion cascade has been divided into sequential steps based on visual assessment of the post-capillary venules during the early stages of acute inflammation. In the absence of inflammation, leukocytes are rarely seen to interact with the vessel wall. After the inflammatory stimulus is applied, leukocytes roll along the post-capillary venules (but not arterioles or small arteries) at velocities distinctly below that of flowing blood. Some rolling cells can be seen to arrest and after a few minutes change shape in apparent response to local chemotactic stimuli. Extravasation into the extravascular tissue follows. Each of these steps requires either upregulation or activation of distinct sets of adhesion molecules [95]. The selectin family of adhesion molecules mediates rolling, the initial capture of leukocytes from the rapidly flowing bloodstream to the blood vessel, before their firm adhesion and diapedesis at sites of tissue injury and inflammation [96]. Although rolling appears to be a prerequisite for eventual firm adherence to blood vessels under conditions of flow, selectin-dependent adhesion of leukocytes does not lead to firm adhesion and transmigration, unless another set of adhesion molecules, the integrins, is engaged. Integrins are a family of heterodimeric membrane glycoproteins that consist of an α and a β subunit; these subunits are associated through noncovalent bonds and transported to the cell surface as a complex [97]. IL-8 and possibly other neutrophil chemoattractant chemokines synthesized by microvascular endothelial cells, may play an important role in leukocyte recruitment and activation in the infarcted myocardium beyond their chemotactic properties [98]. IL-8 induces the neutrophil respiratory burst and granule release, and enhances cellular adhesion, a β2 integrin-dependent event. Recent experiments suggested that both mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) are activated in response to IL-8 stimulation, and that these may represent independent pathways for β2 integrin activation in neutrophils [98]. It appears that neutrophils may need to sample immobilized IL-8 molecules presented by the vessel wall before forming a sufficient number of high avidity β2 integrin bonds for firm adhesion [99]. Obviously, neutrophil recruitment in the infarcted myocardium may require the participation of non-chemokine associated mechanisms such as activated complement, leukotrienes and platelet activating factor (PAF).

Role of chemokines in mononuclear cell recruitment and fibrous tissue deposition

Despite the potentially injurious effects of the inflammatory response in the ischemic myocardium, both experimental and clinical evidence demonstrate that an open infarct vessel promotes repair even when reperfusion occurs when no myocardial tissue can be salvaged [100, 101]. The role of reperfusion-induced inflammation in the repair process has been suggested in several experimental models [101]. Infiltrating mononuclear cells and mast cells appear to orchestrate the cardiac repair process through a complex cascade involving cytokines and growth factors [41, 61, 102, 103]. Mononuclear cells infiltrate the infarcted myocardium in the first few hours of reperfusion. Evidence suggests that the CC chemokine MCP-1 may be an important factor responsible for mononuclear cell recruitment. Studies in a canine model of experimental myocardial infarction indicated that monocyte chemotactic activity in the first h after reperfusion was wholly attributable to C5a [62]. After 3 h of reperfusion, monocyte chemotactic activity in the cardiac lymph was largely dependent on MCP-1 acting in concert with TGF-β1 [62]. MCP-1 mRNA and protein was rapidly upregulated in the venular endothelium of ischemic myocardial segments. In addition to its potential effects on mononuclear cell recruitment, MCP-1 may also regulate macrophage activation and phenotype [104] and may affect cytokine expression in the infarct. MCP-1 is crucial for development of Th2 responses and lymph node cells from immunized MCP-1 –/– mice show markedly decreased IL-10 expression, despite the absence of a defect in T cell trafficking [105]. In healing infarcts T-cell derived IL-10 may be important in inhibiting expression of pro-inflammatory cytokines and in regulating extracellular matrix remodeling [61].

The mononuclear cell chemoattractants MIP-1α and MIP-1β are also markedly induced in myocardial infarcts, however their contribution in recruiting mononuclear cells remains unknown. It is possible that different chemokines may selectively recruit specific subsets of monocytes and

lymhocytes in the injured myocardium affecting distinct pathways of the inflammatory response. Studies using animals deficient in MCP-1 and MIP-1α are currently in progress in our laboratory and may elucidate the specific role of these chemokines in infarct healing.

The role of the chemokines in infarct angiogenesis

Formation of new blood vessels is critical for supplying the healing infarct, with oxygen and nutrients necessary to sustain metabolism. Angiogenesis is dependent on a complex interaction between extracellular matrix, endothelial cells and pericytes in response to an imbalance in the presence of angiogenic as compared to angiostatic factors in the local environment [106]. Myocardial ischemia is associated with synthesis and early release of potent angiogenic factors, such as vascular endothelial growth factor (VEGF) [107, 108] and basic fibroblast growth factor (bFGF) [109]. Chemokine involvement in infarct angiogenesis should be considered as part of the dynamic interaction between angiogenic and angiostatic factors in various stages of healing. Members of the CXC chemokine family may play a role in the regulation of angiogenesis [13, 110]. CXC chemokines behave as either angiogenic or angiostatic depending on the presence of the 'ELR' motif. ELR positive CXC chemokines, such as IL-8, are potent angiogenic factors, inducing both in vitro endothelial chemotaxis and in vivo corneal neovascularization [50]. In contrast, the ELR negative chemokines, such as IP-10, demonstrate robust angiostatic effects in the presence of IL-8 or basic FGF [111, 112].

We have recently demonstrated that IP-10 in induced in both canine [33] and murine [90] myocardial infarcts. IP-10 mRNA expression peaked after 1–3 h of reperfusion and was markedly decreased by 10 h of reperfusion. IP-10 mRNA and protein was localized in the venular endothelium of ischemic myocardial segments. By 24 h of reperfusion neither IP-10 mRNA nor protein were detected. We suggest that IP-10, a weak mononuclear cell chemoattractant, may have a unique role in infarct healing preventing premature granulation tissue formation until the wound is debrided and a fibrin-based temporary matrix, necessary to support ingrowth of granulation tissue is formed. Ongoing functional studies using antibody neutralization and IP-10 KO animals [113] will test this intriguing hypothesis.

MCP-1 may also have an active role in infarct angiogenesis. MCP-1 is a direct mediator of angiogenesis, and endothelial cells express functional CCR2 receptors [83], [114]. In addition, MCP-1 (–/–) mice exhibit delayed wound angiogenesis demonstrating lower capillary density than their wildtype littermates [115]. Studies using MCP-1 deficient mice may elucidate the potential role of MCP-1 in neovascular formation after experimental myocardial infarction.

Do chemokines regulate stem cell recruitment in the infarcted myocardium?

Cardiomyocytes are thought to be terminally differentiated cells. However, recent reports suggested that myocytes may in some cases re-enter the cell cycle. Beltrami and coworkers identified events characteristic of cell division such as the formation of the mitotic spindles and contractile rings, karyokinesis, and cytokinesis in myocytes from patients who died from myocardial infarction [116]. Four percent of myocyte nuclei from regions adjacent to the infarct exhibited expression of Ki-67, a nuclear antigen associated with cell division. These proliferating cells may originate from cardiac resident stem cells or circulating stem cells that home to the heart and may expand producing a differentiated progeny upon stimulation. Although recruitment of bone marrow-derived endothelial progenitor cells may be important for neovascularization, the concept of myocardial regeneration through stem cell infiltration has not been universally accepted [117–119]. Recent studies suggested that bone marrow cells can induce myocardial regeneration after infarction suggesting that blood-borne cells may differentiate into cardiomyocytes [70, 120]. The mechanisms involved in homing of primitive stem cells remain unknown, however inflammatory mediators such as SCF, a factor highly induced in infarcts [103] and certain chemokines may be important in stem cell recruitment. A recent study using a rat model indicated that the CXC chemokine SDF-1a was sufficient to induce therapeutic stem cell homing to the infarcted myocardium [72]. Although, therapeutic approaches targeting stem cells are an important long-term goal in treatment of myocardial infarction, regeneration of myocardium using our current expertise may not be a realistic target, considering the lack of understanding of the mechanisms involved in stem cell homing and differentiation.

Downregulation of chemokine synthesis and resolution of inflammation may be crucial for effective repair

Induction of chemokines, cytokine upregulation, and leukocyte infiltration occur in the inflammatory phase of myocardial infarction and may be important in clearance of the wound from dead cells and debris. However, this acute localized inflammatory response is transient, and its suppression is rapidly followed by fibrous tissue deposition (Fig. 2) [90]. During the proliferative phase of healing, chemokine synthesis and leukocyte recruitment are suppressed, ensuring the transition from inflammation to fibrosis. Inhibition of chemokine synthesis after a dramatic early peak may be crucial for the repair process, preventing prolonged expression of inflammatory mediators in the healing infarct, and continuous leukocyte recruitment and injury. The mechanisms responsible for inflammatory gene downregulation and resolution of the inflammatory response in healing wounds remain poorly understood. Our previous work using a canine model of reperfused infarction suggested IL-10 [61] and TGF-β [33] as potentially important mediators in the resolution of the post-infarction inflammatory response. IL-10 appears to play a role in IL-6 downregulation after infarction [61]. TGF-β, but not IL-10, inhibited cytokine-induced chemokine expression in canine venous endothelial cells [33], suggesting that these inhibitory factors may have distinct roles in regulating the inflammatory process. In addition, TGF-β orchestrates fibroblast-mediated responses and may be important for induction of a wide variety of fibrosis-associated genes. Because of the diversity of its functional

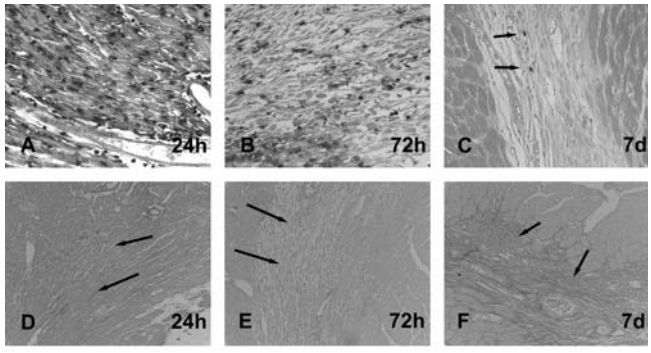

Fig. 2. Resolution of the inflammatory infiltrate is followed by fibrous tissue deposition in murine myocardial infarcts. A–C. Immunohistochemical staining with the antibody F4/80 identifies monocyte/macrophages in reperfused mouse infarcts. Mononuclear cell density peaks after 24 h of reperfusion (A), but decreases significantly after 72 h (B). After 7 days of reperfusion a relatively small number of monocytic cells (arrows) is found in the mouse infarct. Inflammatory leukocyte infiltration is preceded by transient chemokine mRNA induction, that peaks after 6h of reperfusion (Ref. 90). D–F Staining with sirius red identifies collagen fibers in the infarct. After 24 h of reperfusion inflammatory leukocytes (arrows) infiltrate the infarcted area (D). After 72 h highly cellular granulation tissue is formed (arrows) replacing dead cardiomyocytes, however little collagen staining is noted (E). After 7 days of reperfusion, there is extensive deposition of collagen in the healing infarct (F- arrows). Note that reperfused mouse infarcts exhibit an accelerated time course of healing compared with large animal models.

effects, TGF-β may serve as the 'master switch', responsible for the transition from acute inflammation to fibrosis.

Expression of chemokines after a brief non-lethal ischemic insult. Implications for the pathogenesis of ischemic cardiomyopathy

Reperfused infarction is accompanied by cellular necrosis and results in robust expression of chemokines and inflammatory leukocyte recruitment. In order to better understand the response of the heart to injurious stimuli, we asked whether brief ischemic insults that do not result in cardiomyocyte necrosis are sufficient to induce chemokine upregulation in the myocardium. We have recently demonstrated that a single episode of brief non-lethal myocardial ischemia (15 min) followed by reperfusion induces chemokine synthesis in a canine [34] and a murine model [43]. However, in this situation, the modest and transient chemokine upregulation is not accompanied by significant inflammatory cell infiltration. In both the canine and murine model of brief myocardial ischemia, chemokine upregulation is dependent on reactive oxygen generation. Because patients with chronic ischemic heart disease often exhibit recurrent brief ischemic episodes in the absence of myocardial infarction, we examined the effects of repetitive brief ischemia in the murine model. After 3–5 days of repetitive brief ischemia and reperfusion the mouse myocardium demonstrated significant MCP-1 upregulation and macrophage infiltration. Chemokine expression decreases after 7 days of repetitive occlusion, and suppression of the inflammatory response is followed by extensive interstitial fibrosis and left ventricular dysfunction in the absence of a completed infarction [121]. Antibody neutralization experiments indicated that MCP-1 is critical for development of fibrosis in this model [122]. The mechanism responsible for chemokine repression and transition from inflammation to fibrosis is an area of active investigation in our laboratory. It is tempting to hypothesize that TGF-β may be activated in the myocardium suppressing chemokine synthesis and inflammatory leukocyte infiltration. Because of the pro-fibrotic effects of TGF-β, suppression of inflammation may also result in development of fibrosis and dysfunction. These concepts may be relevant to the pathogenesis of chronic ischemic cardiomyopathy. We have recently demonstrated that in patients with chronic ischemic cardiomyopathy, dysfunctional myocardial segments with recovery of function following surgical revascularization had increased inflammatory leukocyte recruitment and MCP-1 expression, compared with irreversibly dysfunctional segments [35]. These findings suggest that chronic ischemic cardiomyopathy is a continuous process [123]. At an early stage induction of inflammatory mediators leads to recruitment of leukocytes in the myocardium. However, acute inflammation may activate endogenous inhibitory factors, such as TGF-β, which may suppress the inflammatory process, but also stimulate fibrosis-associated genes, leading to fibrous tissue deposition and irreversible dysfunction. In contrast to infarction, where chemokine expression may play an important role in granulation tissue formation and healing, the cardiomyopathic process is associated with a maladaptive inflammatory response that results in fibrosis of non-lethally injured myocardium.

Conclusions

Faulty healing and adverse post-infarction remodeling is the leading cause of heart failure and death in patients surviving acute myocardial infarction. Left ventricular remodeling after myocardial infarction in part reflects the magnitude of the initial ischemic change, but is also dependent on the efficiency of the healing process. Chemokines may have a crucial role in infarct healing through effects on both hematopoietic and resident cells. In addition, suppression of the chemokine response is important for the transition to fibrous tissue deposition. Understanding the mechanisms responsible for chemokine downregulation and resolution of the inflammatory infiltrate is important in order to select specific therapeutic targets to optimize healing and cardiac repair.

MCP-1 appears to be an important mediator in the pathogenesis of ischemic cardiomyopathy in both human myocardial tissue and a murine model of brief repetitive ischemia and reperfusion associated with interstitial fibrotic cardiomyopathy. In this situation, a chemokine-driven inflammatory response is triggered in the absence of cellular necrosis, and may play a significant role in the pathogenesis and progression of fibrosis. Hence, MCP-1 inhibition may be an interesting approach in the treatment of chronic ischemic cardiomyopathy.

Acknowledgements. This work was supported by National Institutes of Health grant HL-42550, a grant from the American Heart Association, Texas affiliate, the DeBakey Heart Center, and the Curtis Hankamer Research Fund. The author wishes to thank Concepcion Mata and Sharon Malinowski for their expert secretarial assistance in preparing the manuscript.

References

[1] Olson TS, Ley K. Chemokines and chemokine receptors in leukocyte trafficking. Am J Physiol Regul Integr Comp Physiol 2002; 283: R7–28.
[2] Rollins BJ. Chemokines. Blood 1997; 90: 909–28.
[3] Mackay CR. Chemokines: immunology's high impact factors. Nat Immunol 2001; 2: 95–101.
[4] Baggiolini M, Dewald B, Moser B. Human chemokines: an update. Annu Rev Immunol 1997; 15: 675–705.
[5] Baggiolini M. Chemokines in pathology and medicine. J Intern Med 2001; 250: 91–104.
[6] Sasayama S, Okada M, Matsumori A. Chemokines and cardiovascular diseases. Cardiovasc Res 2000; 45: 267–9.
[7] Wang JM, Su S, Gong W, Oppenheim JJ. Chemokines, receptors, and their role in cardiovascular pathology. Int J Clin Lab Res 1998; 28: 83–90.
[8] Clark-Lewis I, Kim KS, Rajarathnam K, Gong JH, Dewald B, Moser B et al. Structure-activity relationships of chemokines. J Leukoc Biol 1995; 57: 703–11.
[9] Gerard C, Rollins BJ. Chemokines and disease. Nat Immunol 2001; 2: 108–15.
[10] Moser B, Loetscher P. Lymphocyte traffic control by chemokines. Nat Immunol 2001; 2: 123–8.
[11] Middleton J, Patterson AM, Gardner L, Schmutz C, Ashton BA. Leukocyte extravasation: chemokine transport and presentation by the endothelium. Blood 2002; 100: 3853–60.
[12] Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA Jr et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 1999; 398: 718–23.
[13] Strieter RM, Polverini PJ, Arenberg DA, Walz A, Opdenakker G, Van Damme J et al. Role of C-X-C chemokines as regulators of angiogenesis in lung cancer. J Leukoc Biol 1995; 57: 752–62.
[14] Zlotnik A, Morales J, Hedrick JA. Recent advances in chemokines and chemokine receptors. Crit Rev Immunol 1999; 19: 1–47.
[15] Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity 2000; 12: 121–7.
[16] Shin WS, Szuba A, Rockson SG. The role of chemokines in human cardiovascular pathology: enhanced biological insights. Atherosclerosis 2002; 160: 91–102.
[17] Mach F. The role of chemokines in atherosclerosis. Curr Atheroscler Rep 2001; 3: 243–51.
[18] Haskell CA, Hancock WW, Salant DJ, Gao W, Csizmadia V, Peters W et al. Targeted deletion of CX(3)CR1 reveals a role for fractalkine in cardiac allograft rejection. J Clin Invest 2001; 108: 679–88.
[19] Yun JJ, Whiting D, Fischbein MP, Banerji A, Irie Y, Stein D et al. Combined blockade of the chemokine receptors CCR1 and CCR5 attenuates chronic rejection. Circulation 2004; 109: 932–7.
[20] Takeya M, Yoshimura T, Leonard EJ, Takahashi K. Detection of monocyte chemoattractant protein-1 in human atherosclerotic lesions by an anti-monocyte chemoattractant protein-1 monoclonal antibody. Hum Pathol 1993; 24: 534–9.
[21] Apostolopoulos J, Davenport P, Tipping PG. Interleukin-8 production by macrophages from atheromatous plaques. Arterioscler Thromb Vasc Biol 1996; 16: 1007–12.
[22] Mach F, Sauty A, Iarossi AS, Sukhova GK, Neote K, Libby P et al. Differential expression of three T lymphocyte-activating CXC chemokines by human atheroma-associated cells. J Clin Invest 1999; 104: 1041–50.
[23] Abi-Younes S, Sauty A, Mach F, Sukhova GK, Libby P, Luster AD. The stromal cell-derived factor-1 chemokine is a potent platelet agonist highly expressed in atherosclerotic plaques. Circ Res 2000; 86: 131–8.
[24] Haque NS, Zhang X, French DL, Li J, Poon M, Fallon JT et al. CC chemokine I-309 is the principal monocyte chemoattractant induced by apolipoprotein(a) in human vascular endothelial cells. Circulation 2000; 102: 786–92.
[25] Wong BW, Wong D, McManus BM. Characterization of fractalkine (CX3CL1) and CX3CR1 in human coronary arteries with native atherosclerosis, diabetes mellitus, and transplant vascular disease. Cardiovasc Pathol 2002; 11: 332–8.
[26] Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell 1998; 2: 275–81.
[27] Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 1998; 394: 894–7.
[28] Lesnik P, Haskell CA, Charo IF. Decreased atherosclerosis in CX3CR1-/- mice reveals a role for fractalkine in atherogenesis. J Clin Invest 2003; 111: 333–40.
[29] Russell ME, Adams DH, Wyner LR, Yamashita Y, Halnon NJ, Karnovsky MJ. Early and persistent induction of monocyte chemoattractant protein 1 in rat cardiac allografts. Proc Natl Acad Sci USA 1993; 90: 6086–90.
[30] Hancock WW, Wang L, Ye Q, Han R, Lee I. Chemokines and their receptors as markers of allograft rejection and targets for immunosuppression. Curr Opin Immunol 2003; 15: 479–86.
[31] Kumar AG, Ballantyne CM, Michael LH, Kukielka GL, Youker KA, Lindsey ML et al. Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circulation 1997; 95: 693–700.
[32] Kukielka GL, Smith CW, LaRosa GJ, Manning AM, Mendoza LH, Daly TJ et al. Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. J Clin Invest 1995; 95: 89–103.
[33] Frangogiannis NG, Mendoza LH, Lewallen M, Michael LH, Smith CW, Entman ML. Induction and suppression of interferon-inducible protein 10 in reperfused myocardial infarcts may regulate angiogenesis. FASEB J 2001; 15: 1428–30.
[34] Lakshminarayanan V, Lewallen M, Frangogiannis NG, Evans AJ, Wedin KE, Michael LH et al. Reactive oxygen intermediates induce monocyte chemotactic protein-1 in vascular endothelium after brief ischemia. Am J Pathol 2001; 159: 1301–11.
[35] Frangogiannis NG, Shimoni S, Chang SM, Ren G, Shan K, Aggeli C et al. Evidence for an active inflammatory process in the hibernating human myocardium. Am J Pathol 2002; 160: 1425–33.
[36] Rossen RD, Michael LH, Kagiyama A, Savage HE, Hanson G, Reisberg MA et al. Mechanism of complement activation after coronary artery occlusion: evidence that myocardial ischemia in dogs causes release of constituents of myocardial subcellular origin that complex with human C1q in vivo. Circ Res 1988; 62: 572–84.
[37] Lefer DJ, Granger DN. Oxidative stress and cardiac disease. Am J Med 2000; 109: 315–23.
[38] Granger DN. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am J Physiol 1988; 255: H1269–75.
[39] Stancovski I, Baltimore D. NF-kappaB activation: the I kappaB kinase revealed? Cell 1997; 91: 299–302.
[40] Frangogiannis NG, Lindsey ML, Michael LH, Youker KA, Bressler RB, Mendoza LH et al. Resident cardiac mast cells degranulate and release preformed TNF-alpha, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation 1998; 98: 699–710.
[41] Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002; 53: 31–47.
[42] Parissis JT, Adamopoulos S, Venetsanou KF, Mentzikof DG, Karas SM, Kremastinos DT. Serum profiles of C-C chemokines in acute myocardial infarction: possible implication in postinfarction left ventricular remodeling. J Interferon Cytokine Res 2002; 22: 223–9.
[43] Nossuli TO, Frangogiannis NG, Knuefermann P, Lakshminarayanan V, Dewald O, Evans AJ et al. Brief murine myocardial I/R induces chemokines in a TNF-alpha-independent manner: role of oxygen radicals. Am J Physiol Heart Circ Physiol 2001; 281: H2549–58.

[44] Maekawa N, Wada H, Kanda T, Niwa T, Yamada Y, Saito K et al. Improved myocardial ischemia/reperfusion injury in mice lacking tumor necrosis factor-alpha. J Am Coll Cardiol 2002; 39: 1229–35.
[45] Kilgore KS, Park JL, Tanhehco EJ, Booth EA, Marks RM, Lucchesi BR. Attenuation of interleukin-8 expression in C6-deficient rabbits after myocardial ischemia/reperfusion. J Mol Cell Cardiol 1998; 30: 75–85.
[46] McKee CM, Penno MB, Cowman M, Burdick MD, Strieter RM, Bao C et al. Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44. J Clin Invest 1996; 98: 2403–13.
[47] Taylor KR, Trowbridge JM, Rudisill JA, Termeer CC, Simon JC, Gallo RL. Hyaluronan fragments stimulate dermal endothelial recognition of injury through TLR4. J Biol Chem 2004.
[48] Clark-Lewis I, Schumacher C, Baggiolini M, Moser B. Structure-activity relationships of interleukin-8 determined using chemically synthesized analogs. Critical role of NH2-terminal residues and evidence for uncoupling of neutrophil chemotaxis, exocytosis, and receptor binding activities. J Biol Chem 1991; 266: 23128–34.
[49] Zeilhofer HU, Schorr W. Role of interleukin-8 in neutrophil signaling. Curr Opin Hematol 2000; 7: 178–82.
[50] Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science 1992; 258: 1798–801.
[51] Ivey CL, Williams FM, Collins PD, Jose PJ, Williams TJ. Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit. Evidence for a role for C5a and interleukin-8. J Clin Invest 1995; 95: 2720–8.
[52] Boyle EM Jr, Kovacich JC, Hebert CA, Canty TG Jr, Chi E, Morgan EN et al. Inhibition of interleukin-8 blocks myocardial ischemia-reperfusion injury. J Thorac Cardiovasc Surg 1998; 116: 114–21.
[53] Anisowicz A, Bardwell L, Sager R. Constitutive overexpression of a growth-regulated gene in transformed Chinese hamster and human cells. Proc Natl Acad Sci USA 1987; 84: 7188–92.
[54] Cochran BH, Reffel AC, Stiles CD. Molecular cloning of gene sequences regulated by platelet-derived growth factor. Cell 1983; 33: 939–47.
[55] Chandrasekar B, Smith JB, Freeman GL. Ischemia-reperfusion of rat myocardium activates nuclear factor-KappaB and induces neutrophil infiltration via lipopolysaccharide-induced CXC chemokine. Circulation 2001; 103: 2296–302.
[56] Colletti LM, Kunkel SL, Walz A, Burdick MD, Kunkel RG, Wilke CA et al. Chemokine expression during hepatic ische-mia/reperfusion-induced lung injury in the rat. The role of epithelial neutrophil activating protein. J Clin Invest 1995; 95: 134–41.
[57] Tarzami ST, Miao W, Mani K, Lopez L, Factor SM, Berman JW et al. Opposing effects mediated by the chemokine receptor CXCR2 on myocardial ischemia-reperfusion injury: recruitment of potentially damaging neutrophils and direct myocardial protection. Circulation 2003; 108: 2387–92.
[58] Strieter RM, Polverini PJ, Kunkel SL, Arenberg DA, Burdick MD, Kasper J et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 1995; 270: 27348–57.
[59] Shiraha H, Glading A, Gupta K, Wells A. IP-10 inhibits epidermal growth factor-induced motility by decreasing epidermal growth factor receptor-mediated calpain activity. J Cell Biol 1999; 146: 243–54.
[60] Frangogiannis NG, Mendoza LH, Smith CW, Michael LH, Entman ML. Induction of the synthesis of the C-X-C chemokine interferon-gamma-inducible protein-10 in experimental canine endotoxemia. Cell Tissue Res 2000; 302: 365–76.
[61] Frangogiannis NG, Mendoza LH, Lindsey ML, Ballantyne CM, Michael LH, Smith CW et al. IL-10 is induced in the reperfused myocardium and may modulate the reaction to injury. J Immunol 2000; 165: 2798–808.
[62] Birdsall HH, Green DM, Trial J, Youker KA, Burns AR, Mac Kay CR et al. Complement C5a, TGF-beta 1, and MCP-1, in sequence, induce migration of monocytes into ischemic canine myocardium within the first one to five hours after reperfusion. Circulation 1997; 95: 684–92.
[63] Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996; 382: 635–8.
[64] Salvucci O, Yao L, Villalba S, Sajewicz A, Pittaluga S, Tosato G. Regulation of endothelial cell branching morphogenesis by endogenous chemokine stromal-derived factor-1. Blood 2002; 99: 2703–11.
[65] Salcedo R, Wasserman K, Young HA, Grimm MC, Howard OM, Anver MR et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1alpha. Am J Pathol 1999; 154: 1125–35.
[66] Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J Exp Med 1997; 185: 111–20.
[67] Jo DY, Rafii S, Hamada T, Moore MA. Chemotaxis of primitive hematopoietic cells in response to stromal cell-derived factor-1. J Clin Invest 2000; 105: 101–11.
[68] Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 1999; 283: 845–8.
[69] Pillarisetti K, Gupta SK. Cloning and relative expression analysis of rat stromal cell derived factor-1 (SDF-1)1: SDF-1 alpha mRNA is selectively induced in rat model of myocardial infarction. Inflammation 2001; 25: 293–300.
[70] Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410: 701–5.
[71] Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001; 107: 1395–402.
[72] Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003; 362: 697–703.
[73] Rollins BJ. Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease. Mol Med Today 1996; 2: 198–204.
[74] Gu L, Tseng SC, Rollins BJ. Monocyte chemoattractant protein-1. Chem Immunol 1999; 72: 7–29.
[75] Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 1991; 88: 1121–7.
[76] Gu L, Rutledge B, Fiorillo J, Ernst C, Grewal I, Flavell R et al. In vivo properties of monocyte chemoattractant protein-1. J Leukoc Biol 1997; 62: 577–80.
[77] Koch AE, Kunkel SL, Harlow LA, Johnson B, Evanoff HL, Haines GK et al. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. J Clin Invest 1992; 90: 772–9.
[78] Wang X, Yue TL, Barone FC, Feuerstein GZ. Monocyte chemoattractant protein-1 messenger RNA expression in rat ischemic cortex. Stroke 1995; 26: 661–5; discussion 665–6.
[79] Tesch GH, Schwarting A, Kinoshita K, Lan HY, Rollins BJ, Kelley VR. Monocyte chemoattractant protein-1 promotes macrophage-mediated tubular injury, but not glomerular injury, in nephrotoxic serum nephritis. J Clin Invest 1999; 103: 73–80.
[80] Ono K, Matsumori A, Furukawa Y, Igata H, Shioi T, Matsushima K et al. Prevention of myocardial reperfusion injury in rats by an antibody against monocyte chemotactic and activating factor/monocyte chemoattractant protein-1. Lab Invest 1999; 79: 195–203.
[81] Kakio T, Matsumori A, Ono K, Ito H, Matsushima K, Sasayama S. Roles and relationship of macrophages and monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 in the ischemic and reperfused rat heart. Lab Invest 2000; 80: 1127–36.

[82] Tarzami ST, Cheng R, Miao W, Kitsis RN, Berman JW. Chemokine expression in myocardial ischemia: MIP-2 dependent MCP-1 expression protects cardiomyocytes from cell death. J Mol Cell Cardiol 2002; 34: 209–21.
[83] Salcedo R, Ponce ML, Young HA, Wasserman K, Ward JM, Kleinman HK et al. Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. Blood 2000; 96: 34–40.
[84] Gharaee-Kermani M, Denholm EM, Phan SH. Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. J Biol Chem 1996; 271: 17779–84.
[85] Ban K, Ikeda U, Takahashi M, Kanbe T, Kasahara T, Shimada K. Expression of intercellular adhesion molecule-1 on rat cardiac myocytes by monocyte chemoattractant protein-1. Cardiovasc Res 1994; 28: 1258–62.
[86] Hayashidani S, Tsutsui H, Shiomi T, Ikeuchi M, Matsusaka H, Suematsu N et al. Anti-monocyte chemoattractant protein-1 gene therapy attenuates left ventricular remodeling and failure after experimental myocardial infarction. Circulation 2003; 108: 2134–40.
[87] Wolpe SD, Davatelis G, Sherry B, Beutler B, Hesse DG, Nguyen HT et al. Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. J Exp Med 1988; 167: 570–81.
[88] Wolpe SD, Cerami A. Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. FASEB J 1989; 3: 2565–73.
[89] Uguccioni M, D'Apuzzo M, Loetscher M, Dewald B, Baggiolini M. Actions of the chemotactic cytokines MCP-1, MCP-2, MCP-3, RANTES, MIP-1 alpha and MIP-1 beta on human monocytes. Eur J Immunol 1995; 25: 64–8.
[90] Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C et al. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. Am J Pathol 2004; 164: 665–77.
[91] Schall TJ. Biology of the RANTES/SIS cytokine family. Cytokine 1991; 3: 165–83.
[92] Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 1990; 347: 669–71.
[93] Engler RL, Dahlgren MD, Morris DD, Peterson MA, Schmid-Schonbein GW. Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. Am J Physiol 1986; 251: H314–23.
[94] Thelen M, Peveri P, Kernen P, von Tscharner V, Walz A, Baggiolini M. Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. FASEB J 1988; 2: 2702–6.
[95] Ebnet K, Vestweber D. Molecular mechanisms that control leukocyte extravasation: the selectins and the chemokines. Histochem Cell Biol 1999; 112: 1–23.
[96] McEver RP. Selectins: lectins that initiate cell adhesion under flow. Curr Opin Cell Biol 2002; 14: 581–6.
[97] Luscinskas FW, Lawler J. Integrins as dynamic regulators of vascular function. Faseb J 1994; 8: 929–38.
[98] Takami M, Terry V, Petruzzelli L. Signaling pathways involved in IL-8-dependent activation of adhesion through Mac-1. J Immunol 2002; 168: 4559–66.
[99] DiVietro JA, Smith MJ, Smith BR, Petruzzelli L, Larson RS, Lawrence MB. Immobilized IL-8 triggers progressive activation of neutrophils rolling in vitro on P-selectin and intercellular adhesion molecule-1. J Immunol 2001; 167: 4017–25.
[100] Late Assessment of Thrombolytic Efficacy (LATE) study with alteplase 6-24 hours after onset of acute myocardial infarction. Lancet 1993; 342: 759–66.
[101] Richard V, Murry CE, Reimer KA. Healing of myocardial infarcts in dogs. Effects of late reperfusion. Circulation 1995; 92: 1891–901.
[102] Frangogiannis NG, Michael LH, Entman ML. Myofibroblasts in reperfused myocardial infarcts express the embryonic form of smooth muscle myosin heavy chain (SMemb). Cardiovasc Res 2000; 48: 89–100.
[103] Frangogiannis NG, Perrard JL, Mendoza LH, Burns AR, Lindsey ML, Ballantyne CM et al. Stem cell factor induction is associated with mast cell accumulation after canine myocardial ischemia and reperfusion. Circulation 1998; 98: 687–98.
[104] Biswas SK, Sodhi A. In vitro activation of murine peritoneal macrophages by monocyte chemoattractant protein-1: upregulation of CD11b, production of proinflammatory cytokines, and the signal transduction pathway. J Interferon Cytokine Res 2002; 22: 527–38.
[105] Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature 2000; 404: 407–11.
[106] Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med 2000; 6: 389–95.
[107] Li J, Brown LF, Hibberd MG, Grossman JD, Morgan JP, Simons M. VEGF, flk-1, and flt-1 expression in a rat myocardial infarction model of angiogenesis. Am J Physiol 1996; 270: H1803–11.
[108] Hashimoto E, Ogita T, Nakaoka T, Matsuoka R, Takao A, Kira Y. Rapid induction of vascular endothelial growth factor expression by transient ischemia in rat heart. Am J Physiol 1994; 267: H1948–54.
[109] Bernotat-Danielowski S, Sharma HS, Schott RJ, Schaper W. Generation and localisation of monoclonal antibodies against fibroblast growth factors in ischaemic collateralised porcine myocardium. Cardiovasc Res 1993; 27: 1220–8.
[110] Strieter RM, Polverini PJ, Arenberg DA, Kunkel SL. The role of CXC chemokines as regulators of angiogenesis. Shock 1995; 4: 155–60.
[111] Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S et al. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. J Exp Med 1995; 182: 155–62.
[112] Angiolillo AL, Sgadari C, Tosato G. A role for the interferon-inducible protein 10 in inhibition of angiogenesis by interleukin-12. Ann N Y Acad Sci 1996; 795: 158–67.
[113] Hancock WW, Gao W, Csizmadia V, Faia KL, Shemmeri N, Luster AD. Donor-derived IP-10 initiates development of acute allograft rejection. J Exp Med 2001; 193: 975–80.
[114] Weber KS, Nelson PJ, Grone HJ, Weber C. Expression of CCR2 by endothelial cells: implications for MCP-1 mediated wound injury repair and In vivo inflammatory activation of endothelium. Arterioscler Thromb Vasc Biol 1999; 19: 2085–93.
[115] Low QE, Drugea IA, Duffner LA, Quinn DG, Cook DN, Rollins BJ et al. Wound healing in MIP-1alpha(–/–) and MCP-1(–/–) mice. Am J Pathol 2001; 159: 457–63.
[116] Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R et al. Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 2001; 344: 1750–7.
[117] Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004.
[118] Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 2004.
[119] Chien KR. Stem cells: Lost in translation. Nature 2004.
[120] Anversa P, Nadal-Ginard B. Myocyte renewal and ventricular remodelling. Nature 2002; 415: 240–3.
[121] Dewald O, Frangogiannis NG, Zoerlein M, Duerr GD, Klemm C, Knuefermann P et al. Development of murine ischemic cardiomyopathy is associated with a transient inflammatory reaction and depends on reactive oxygen species. Proc Natl Acad Sci USA 2003; 100: 2700–5.
[122] Dewald O, Ren G, Klemm C, Winkelmann K, Koerting A, Taffet G et al. Development of murine fibrotic cardiomyopathy is dependent on Monocyte Chemoattractant Protein 1. J Am Coll Cardiol 2004; 43: 230A.
[123] Frangogiannis NG, Shimoni S, Chang SM, Ren G, Dewald O, Gersch C et al. Active interstitial remodeling: an important process in the hibernating human myocardium. J Am Coll Cardiol 2002; 39: 1468–74.