Chemokines in Vascular Dysfunction and Remodeling

Andreas Schober

Abstract—Vascular remodeling stands for structural changes of the vessel wall in response to various noxious stimuli, which results in reorganization of the vessel wall architecture. Luminal narrowing because of neointima formation and constrictive remodeling leading to hypoperfusion is the most relevant clinical effect. Smooth muscle cell (SMC) accumulation, inflammatory cell recruitment, and endothelial regeneration are the critical parts in obstructive vascular remodeling. Chemokines and chemokine receptors have a great impact on initiating and progressing neointimal formation by controlling each step of the remodeling process. SDF-1α regulates vascular repair by CXCR4-dependent smooth muscle progenitor cell recruitment, which contributes to the maladaptive response to injury. The three distinct chemokine-chemokine receptor pairs MCP-1/CCR2, RANTES/CCR5, and Fractalkine/CX3CR1 direct lesional leukocyte infiltration. In addition MCP-1/CCR2 and Fractalkine/CX3CR1 increase neointimal SMC expansion. In contrast, KC/Gro-α supports endothelial recovery through CXCR2, which attenuates neointima formation. These findings highlight the importance to characterize specific functions of the chemokine network to enable therapeutic intervention. (Arterioscler Thromb Vasc Biol. 2008;28:1950-1959)

Vascular remodeling denotes morphological changes of the vessel wall in response to various noxious stimuli inducing reorganization of the vessel wall structure. This impacts the cross-sectional vessel diameter and the thickness of the arterial wall in every direction; however, luminal narrowing and resulting hypoperfusion are the most detrimental sequelae of vascular remodeling. Hemodynamic stress, mechanical injury, inflammation, or hypoxia are only some of the clinically met triggers for adaptive remodeling of the vessel wall, for instance after percutaneous interventions, heart transplantation, or vein grafting. Morphologically, all three layers of the arterial wall are concurrently affected by neointimal hyperplasia, medial thickening, and adventitial fibrosis attributable to the interaction of leukocyte recruitment, smooth muscle cell (SMC) accumulation, and endothelial recovery. Molecularly, chemokines play an ever-emerging role in vascular repair through guidance of circulating mononuclear cells to the injury site and activation of resident vascular cells. Considering the cell-type specific expression of chemokine receptors and the substantial overlap in ligand-receptor specificity, an interactive network of chemokines and chemokine receptors emerges with enormous plasticity in different types of vascular injury. However, a growing number of chemokine-chemokine receptor pairs with confined effects in vascular diseases have been described. To modulate the maladaptive response in arterial remodeling, it is essential to identify specific therapeutic targets in the chemokine network. The contribution of the chemokine system to arteriosclerotic diseases has been previously reviewed in-depth. This review focuses on most recent findings in regulation and function of distinct chemokine axes constituting specific repair events in the injured artery.

Vascular Remodeling by SDF-1α–Mediated Recruitment of Progenitor Cells

An instant effect of mechanical vascular injury with endothelial denudation and distension of the media is the apoptosis of up to 70% of the medial SMCs. This occurs as early as 30 minutes after balloon injury with a marked decrease of the vessel wall cellularity, correlates with the intensity of the injury, and precedes the proliferative peak in the media. Inhibition of the early medial SMC apoptosis attenuates neointima formation, suggesting apoptosis as a signal for an increased demand in vascular repair to overcome the cellular deficit. In this context, replacement by resident SMCs is impaired requiring a backup repair system by circulating...
progenitor cells. In fact, smooth muscle progenitor cell (SPC) recruitment is increased in injury models with prominent medial cell apoptosis and contributes to intimal thickening after mechanical injury and transplant vasculopathy. Bone marrow (BM)–derived SPCs in the circulation are a major source for neointimal SMCs in vascular repair. In addition, the adventitial tissue harbors SPCs; however, the impact of endogenous adventitial cells to neointimal hyperplasia may vary according to the disease model.

The CXC-chemokine stromal cell-derived factor (SDF)-1α (also known as CXCL12) has been originally purified from the supernatant of BM stromal cells, but is constitutively expressed in various tissues. Compared with other chemokines, SDF-1α selectively induces the migration of BM-derived progenitor cells. The high SDF-1α level in the BM creates a concentration gradient, which retains hematopoietic progenitor cells, and disruption of this SDF-1α gradient causes mobilization of stem cells into the circulation. In contrast to other chemokines, SDF-1α signaling was thought to occur exclusively by the G protein–coupled seven transmembrane receptor (GPCR) CXCR4, because targeted deletion of SDF-1α or CXCR4 in mice results in similar phenotypes, including embryonic lethality. However, CXCR7 (RDC1/Cmkor1) has been identified as another SDF-1α receptor, which forms a heterodimer with CXCR4, thereby regulating GPCR activity. Furthermore, the chemokine-like cytokine macrophage migration inhibitory factor (MIF) is a noncognate ligand for CXCR4 and CXCR2, which stimulates neointimal macrophage infiltration after carotid wire-injury most likely through CXCR2.

After different types of vascular injury, increased SDF-1α expression particularly in SMCs accompanied by a transient rise in SDF-1α plasma levels has been reported. Notably, wire-induced carotid injury in mice causes prominent SDF-1α expression in the media within 24 hours, which remains elevated throughout the vessel wall during neointima formation. In addition, activated platelets release and subsequently present SDF-1α on the surface after vascular injury. The increase in circulating SDF-1α affects the gradient of SDF-1α between the blood and the BM, thus mobilizing Sca-1/Lin− SPC. Further characterization of SPCs revealed that SDF-1α preferentially mobilizes and recruits c-kit+/Sca-1−/Lin− cells to the neointima, which lack the long-term repopulating potential of hematopoietic stem cells and express the PDGF receptor β. The implication of SDF-1α on intimal hyperplasia after wire-injury has been demonstrated in hyperlipidemic ApoE−/− mice. Treatment with a neutralizing SDF-1α antibody reduces the neointimal area and SMC content through inhibition of BM-derived SPC accumulation without affecting macrophage infiltration. Local SDF-1α expression in the injured artery is critical for vascular remodeling, because local gene transfer of a SDF-1α antagonist peptide inhibits neointima formation. ApoE−/− mice after BM reconstitution with fetal hematopoietic stem cells from CXCR4−/− mice show also decreased neointima formation and SMC accumulation, suggesting SDF-1α–dependent SPC recruitment by
CXCR4 expression (Figure 1). Recent evidence indicates that the A2b adenosine receptor (A2bAR) regulates CXCR4 expression in SPCs, because genetic deficiency of A2bAR increases CXCR4 on BM cells and exacerbates neointimal growth after femoral wire injury.45 Chronic pharmacological blockade of CXCR4 using the small molecule antagonist AMD3465 reduces neointima size by 59% and prevents Sca-1+/Lin- cell mobilization after carotid wire injury.46 In contrast, AMD3465 application for 12 weeks in diet-induced atherosclerosis clearly promotes plaque progression and increases lesional neutrophil infiltration.47 There are two reasons to explain this discrepancy. First, the role of SPCs in diet-induced atherosclerosis appears to be vasculoprotective by limiting plaque growth and promoting a stable plaque phenotype.48 Therefore, a decreased lesional SMC content in AMD3465-treated ApoE−/− mice on high cholesterol diet represents an inflammatory plaque type with accelerated growth.47 Second, a deranged neutrophil hemostasis with an increased number of circulating neutrophils is crucial for the proatherogenic effect AMD3465 treatment. In contrast to diet-induced atherosclerosis, neutrophils are only involved in the very early phase of neointima formation after endothelial denudation.49 Thus, neutrophilic leukocytosis induced by chronic CXCR4 inhibition may not contribute significantly to neointimal growth after arterial wire injury.47 CXCR4 inhibition may therefore be a reasonable approach to limit restenosis after percutaneous interventions through systemic (short term) or local (via drug-eluting stents) application. Long term treatment with CXCR4 antagonists, however, may have detrimental sequelae in patients with atherosclerosis.

The role of CXCR7 in neointimal SPC recruitment after vascular injury is unclear; however, CXCR7 and CXCR4 regulate different steps in the therapeutic homing of progenitor cells in mice with acute renal failure.50 Furthermore, MIF may interact with SDF-1α on CXCR4 signaling in vascular remodeling.34,36 Apoptosis of medial SMCs triggers the SPC-mediated vascular repair by the SDF-1α/CXCR4 axis.19 In vitro, microvesicles released from apoptotic SMCs are sufficient to induce SDF-1α secretion from uninjured SMCs.39 Accordingly, wire-induced arterial injury, which causes more extensive apoptosis of medial cells as compared to carotid ligation and periarterial cuff placement, induces the highest neointimal SDF-1α levels.17 Therefore, a high degree of apoptosis signals the demand for vascular repair by circulating progenitor cells via up-regulation of SDF-1α (Figure 1). The molecular mechanism of apoptosis-induced SDF-1α expression remains to be determined. Unlike most other CXC chemokines, the SDF-1α promoter does not include active binding sites for proinflammatory transcription factors, such as nuclear factor (NF)-κB or NF-IL6, and shows a cell-specific regulation pattern.51 The SDF-1α promoter contains two binding sites for the hypoxia-inducible factor-1α (HIF), which control SDF-1α expression in hypoxic endothelial cells through binding to HIF-1α.52 Although the HIF-1 transcriptional system mainly regulates the cellular adaptation to low oxygen supply, nonhypoxic transcriptional and translational upregulation of HIF-1α occurs in SMCs, for instance by thrombin or platelet-derived growth factor (PDGF)-AB.53–55 After wire-induced carotid injury, HIF-1α is rapidly and persistently induced in SMCs and mediates SDF-1α expression in the injured vessel wall.56 Inhibition of HIF-1α upregulation by RNA interference reduces injury-induced neointima formation.56 The tumor suppressor and PI3K/Akt antagonist PTEN (phosphatase and tensin homolog), which decreases neointimal hyperplasia after gene transfer,57 is an important mediator of HIF-1α activity. Mice with a SMC-specific deletion of PTEN show increased HIF-1α-dependent SDF-1α expression with medial hyperplasia through recruiting vascular progenitor cells.58 Treatment with macrophage colony stimulating factor (CSF), a known inducer of HIF-1α, also enhances wire-induced SDF-1α expression in the neointima.40 However, the signaling events connecting SMC apoptosis and HIF-1α activation are not defined (Figure 1).

In experimental models of graft vasculopathy, the alloreactive immune response by T-cells and antibodies causes massive apoptosis of the medial SMCs, and promotes host-derived SPC recruitment in the first weeks after transplantation.59 In analogy to wire injury–induced vascular remodeling, restraint of caspase 3-mediated apoptosis abates neointima formation in transplant arteriosclerosis.60 Although direct evidence for apoptosis-induced SDF-1α expression in graft vasculopathy is lacking, SDF-1α is upregulated in the adventitia and subsequently in the media and neointima of aortic allografts and provokes mobilization and neointimal recruitment of SPCs.52 Furthermore, inhibition of SDF-1α with a blocking antibody reduces intimal thickening and the number of neointimal CXCR4-positive cells.42 In transplanted human hearts, the extent of peritransplant ischemic injury, an important risk factor for cardiac allograft vasculopathy (CAV),61 correlates with increased SDF-1α expression and with the recruitment of recipient-derived SPCs to cardiac blood vessels.62

In summary, vascular injury-induced medial SMC apoptosis activates a universal repair mechanism through SDF-1α-dependent recruitment of SPCs to meet the increased demand for SMC replacement. Thus, the SDF-1α/CXCR4 axis contributes to vascular remodeling, which may be excessive depending on the extent of the injury and then fosters the progression to arterial stenosis.

Chemokines Regulate Inflammatory Cell Infiltration in Vascular Remodeling

Apart from reorganization of SMCs in the injured artery, inflammatory cell recruitment of mainly monocytes and T-cells, owing to an injury-specific cellular immune response, constitutes a general feature in vascular remodeling and promotes disease progression.61,63–65 Chemokines control leukocyte trafficking in various inflammatory diseases including atherosclerosis.4,10 In vascular remodeling, many chemokines, such as MCP-1 (CCL2), RANTES (CCL5), or Fractalkine (CX3CL1), are upregulated in vascular wall cells and cooperate in leukocyte recruitment to the injured artery.8 The proinflammatory phenotype of neointimal SMCs, characterized by increased expression of adhesion molecules and chemokines driven by persistent NF-κB activation, plays a
central role in preserving this inflammatory response. Although the contribution of these chemokines appears redundant, individual chemokine-receptor pairs have been identified, which regulate distinct steps of leukocyte recruitment in vascular remodeling.

**RANTES-Dependent Leukocyte Recruitment**

The CC-chemokine RANTES (Regulated on activation, normally T-expressed, and presumably secreted, also known as CCL5) recruits leukocytes, including T-cells and monocytes, through different chemokine receptors (eg, CCR 1, -3, and -5). RANTES receptors on the other hand bind to several other chemokines, such as MIP-1α/CCL3 (CCR1, CCR5) or eotaxin/CCL11 (CCR3). However, functional specialization of CCR1 and CCR5 in RANTES-induced leukocyte recruitment depending on the oligomerization of RANTES and its heterophilic interaction with PF4 has been described.

In neointimal lesions after wire-induced carotid injury of hyperlipidemic ApoE−/− mice, RANTES has been primarily detected on endothelial cells, where RANTES is deposited after release from activated platelets, and in neointimal SMCs (Figure 2). Treatment with the selective CCR1- and CCR5 antagonist Met-RANTES clearly reduces neointima formation and macrophage infiltration. The transcriptional regulator Y-box binding protein (YB)-1 regulates RANTES expression in neointimal SMCs, which increases monocyte adhesion to SMCs under flow. In vivo knockdown of YB-1 in carotid arteries inhibits RANTES expression in SMCs and impairs neointima formation via reduced macrophage infiltration, similarly to Met-RANTES treatment (Figure 2). Furthermore, YB-1 knockdown in CCR5-deficient mice does not result in reduced neointimal tissue, confirming YB-1 as the crucial ligand in CCR5-mediated intimal hyperplasia. Using genetically targeted mice, CCR5 but not CCR1 appears to be responsible for RANTES-mediated neointimal growth and macrophage infiltration. In addition, CCR5 deficiency diminishes T-cell recruitment and induces a shift toward a T$_{H}2$-type immune response with increased neointimal interleukin (IL)-10 expression. Inhibition of IL-10 reverses the effect of CCR5 deficiency on intimal thickening and macrophage infiltration, implying a protective role of T-cell-derived IL-10 in vascular remodeling. In CCR1−/−/ApoE−/− mice, however, T$_{H}1$-immune response prevails with increased neointimal interferon (IFN)-γ expression. Because IFN-γ inhibition more effectively reduces lesional macrophage content in CCR1−/− as compared to wild-type mice, T$_{H}1$-related proinflammatory mechanisms may counteract the inhibitory effect of CCR1 deficiency on monocyte recruitment in transgenic mice. Besides, these results indicate an important role of the T$_{H}1$/T$_{H}2$ balance in vascular repair after mechanical injury at least in the context of hyperlipidemia, similar to native atherosclerosis.

Platelets contain significant amounts of chemokines, which are released on activation. Platelet-derived RANTES can be immobilized on activated endothelial cells via platelet P-selectin (Figure 2); thus, inducing monocyte arrest and promoting atherogenesis. After wire-injury, RANTES deposition on regenerating endothelial cells covering neointimal lesions is reduced in mice with P-selectin–deficient BM, demonstrating that endothelial immobilization of platelet-derived RANTES occurs in vascular repair (Figure 2). Furthermore, junctional adhesion molecule (JAM)-A has been implicated in endothelial RANTES deposition. JAM-A belongs to the IgG superfamily and is a part of endothelial and epithelial tight junctions. Endothelial JAM-A regulates the transendothelial migration of leukocytes by binding to the β$_3$ integrin lymphocyte function-associated antigen-1. In hyperlipidemic ApoE−/− mice, JAM-A expression is increased in endothelial cells and supports atherogenic leukocyte recruitment. Platelet JAM-A mediates the adhesion of platelet to activated endothelial cells by homophilic interaction with endothelial JAM-A. Interestingly, JAM-A deficiency results in decreased luminal RANTES deposition after wire-induced carotid injury indicating an important role of JAM-A in endothelial deposition of platelet-derived RANTES (Figure 2). Disrupted endothelial RANTES deposition may contribute to the impaired neointima formation and reduced neointimal macrophage content in JAM-A−/− mice (Figure 2).
In allograft vasculopathy, increased RANTES and CCR5 expression have been reported in infiltrating leukocytes, endothelial cells, and intimal SMCs. Treatment with Met-RANTES causes reduced neointimal growth through inhibition of T-cell and monocyte infiltration in a mouse model of CAV. This closely resembles findings in CCR5−/− ApoE−/− mice after carotid wire injury and diet-induced atherosclerosis. Therefore, it can be assumed that the RANTES/CCR5 axis crucially affects T-cell mediated immunity in transplant vasculopathy, characterized by the prevalence of memory Th1 cells, for example by regulating IL-10 synthesis.

Table 1. Experimental Studies on the Role of MCP-1 and CCR2 in Vascular Remodeling

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Hyperlipidemia</th>
<th>Vascular Injury</th>
<th>Effect</th>
<th>Mechanism</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2−/− mice</td>
<td>−</td>
<td>Wire-injury of the femoral artery</td>
<td>Neointima 61% ↓</td>
<td>SMC proliferation ↓</td>
<td>101</td>
</tr>
<tr>
<td>CCR2−/−/ApoE−/− mice</td>
<td>+</td>
<td>Wire-injury of the carotid artery</td>
<td>Neointima 47% ↓</td>
<td>Macrophages ↓</td>
<td>94</td>
</tr>
<tr>
<td>Early monocyte adhesion</td>
<td>↓</td>
<td>Stent placement on femoral artery</td>
<td>Neointima 60% ↓</td>
<td>Macrophages ↓</td>
<td>96</td>
</tr>
<tr>
<td>Monkey, Anti human CCR2 Ab</td>
<td>−</td>
<td>Balloon injury or stenting off the iliac artery</td>
<td>Balloon: no effect</td>
<td>Inflammation ↓</td>
<td>95</td>
</tr>
<tr>
<td>Mice and Monkeys, gene transfer of 7ND</td>
<td>−</td>
<td>Cuff placement on femoral artery</td>
<td>Neointima 60% ↓</td>
<td>Macrophages ↓</td>
<td>96</td>
</tr>
<tr>
<td>Rabbit, gene transfer of 7ND</td>
<td>+</td>
<td>Balloon injury of the carotid artery</td>
<td>Neointima 40% ↓</td>
<td>Macrophages ↓</td>
<td>97</td>
</tr>
<tr>
<td>Rat, MCP-1 Ab</td>
<td>−</td>
<td>Balloon injury of the carotid artery</td>
<td>Neointima 56% ↓</td>
<td>SMC content ↓</td>
<td>93</td>
</tr>
<tr>
<td>Rabbit and Monkey, 7ND gene eluting stent</td>
<td>+</td>
<td>Stenting of the iliac or femoral artery</td>
<td>Neointima 60% ↓</td>
<td>SMC proliferation ↓</td>
<td>93</td>
</tr>
<tr>
<td>Rabbit and Monkey, 7ND gene transfer</td>
<td>+</td>
<td>Stenting of the iliac artery</td>
<td>Neointima 44% ↓ (rabbit) and 28% ↓ (monkey)</td>
<td>Macrophages ↓</td>
<td>99</td>
</tr>
<tr>
<td>Rat, MCP-1 Ab</td>
<td>−</td>
<td>Balloon injury of the carotid artery</td>
<td>Neointima 56% ↓</td>
<td>SMC content ↓</td>
<td>93</td>
</tr>
<tr>
<td>Rabbit and Monkey, 7ND gene transfer</td>
<td>+</td>
<td>Stenting of the iliac or femoral artery</td>
<td>Neointima 29% ↓ (rabbit) and 28% ↓ (monkey)</td>
<td>Macrophages ↓</td>
<td>99</td>
</tr>
<tr>
<td>Rat, MCP-1 Ab</td>
<td>−</td>
<td>Stenting of the iliac artery</td>
<td>Neointima 50% ↓</td>
<td>Macrophages ↓</td>
<td>100</td>
</tr>
<tr>
<td>Rat, MCP-1 Ab</td>
<td>−</td>
<td>Vein graft</td>
<td>Neointima 51% ↓</td>
<td>SMC proliferation ↓</td>
<td>104</td>
</tr>
<tr>
<td>Rat, MCP-1 Ab</td>
<td>−</td>
<td>Autologous vein graft</td>
<td>Neointima 65% ↓</td>
<td>Macrophages ↓</td>
<td>105</td>
</tr>
<tr>
<td>Mice, gene transfer of 7ND</td>
<td>−</td>
<td>Cardiac transplantation</td>
<td>Neointima 39% ↓</td>
<td>Leukocytes ↓</td>
<td>107</td>
</tr>
<tr>
<td>Mouse, gene transfer of 7ND</td>
<td>−</td>
<td>Monocrotaline-induced PH</td>
<td>Media 29% ↓</td>
<td>Monocytes ↓</td>
<td>110</td>
</tr>
<tr>
<td>CCR2−/− mice</td>
<td>−</td>
<td>Angiotensin II−induced hypertension</td>
<td>Wall thickness 65% ↓</td>
<td>Macrophages ↓</td>
<td>117</td>
</tr>
<tr>
<td>Mice with leukocyte-specific CCR2 deletion</td>
<td>−</td>
<td>Angiotensin II−induced hypertension</td>
<td>Wall thickness ↓</td>
<td>Macrophages ↓</td>
<td>118</td>
</tr>
</tbody>
</table>

MCP-1/CCR2 Is Important in Monocyte Homing

The role of the CC-chemokine monocyte chemotactic protein (MCP)-1 (CCL2) and its receptor CCR2 in neointima formation have been extensively studied in experimental models of mechanical vascular injury (Table 1). MCP-1 expression in the injured artery is induced within hours in SMCs with a subsequent increase in the circulation. Because the up-regulation of MCP-1 is transient, its contribution to neointima formation is mainly confined to the early phase. Inhibition of the MCP-1/CCR2 axis quite uniformly reduces intimal hyperplasia in different animal models of arterial injury. However, injury models with a prominent inflammatory response, for example periarterial cuff placement, stent implantation, or endothelial denudation in hyperlipidemic animals, appear to favor MCP-1/CCR2-mediated leukocyte infiltration.
MCP-1 induces transendothelial migration, but not shear-resistant arrest of monocytes on activated endothelial cells. This difference arises from the lack of surface immobilization of MCP-1 on endothelial cells, a prerequisite for chemokine-induced stable adhesion. In contrast, MCP-1 released from medial SMCs is effectively immobilized on platelets adhering to injured arteries.

Gene transfer of a CCR2 antagonist (N-terminal deletion mutant of MCP-1, 7ND) greatly reduces vein graft thickening in different animal models, where infiltrating macrophages express high levels of MCP-1. Although the mechanism of neointima reduction in vein grafts remains equivocal, monocyte recruitment and neointimal cell proliferation are significantly suppressed by inhibition of the MCP-1/CCR2 axis (Table 1). In CAV, MCP-1 is upregulated in arterioles and infiltrating monocytes. Gene transfer of 7ND reduces intimal hyperplasia and the recruitment of CCR2-positive macrophages into graft coronary arteries (Table 1).

Vascular obstruction of pulmonary arterioles through medial and adventitial thickening and a hypoxia-induced inflammatory response within the vessel wall characterizes vascular remodeling in pulmonary hypertension (PH). In patients with idiopathic PH, MCP-1 is upregulated in pulmonary endothelial cells and perivascular leukocytes. Blocking MCP-1 or CCR2 in animal models of PH abates medial thickening and improves right ventricular pressures.

Vascular remodeling contributing to systemic arterial hypertension consists of reduced vessel diameters and medial thickening accelerated by macrophage infiltration. MCP-1 is upregulated in the vessel wall of hypertensive animals by angiotensin II and mechanical stress. In CCR2-deficient mice, hypertension-induced macropage infiltration and vascular hypertrophy are significantly reduced. Similarly, mice with CCR2-deficient leukocytes display a blunted response to angiotensin II regarding vascular inflammation and aortic wall thickening.

**Fractalkine/CX3CR1 Axis Induce Inflammation and SMC Proliferation**

Fractalkine (CX3CL1) is a structurally distinct chemokine, which exists in a membrane-bound or soluble form after shedding from the cell surface. The transmembrane protein supports integrin-independent leukocyte adhesion, whereas the soluble form of fractalkine has a potent chemoattractant activity. Both effects are mediated through CX3CR1, which is involved in atherogenic monocyte recruitment independently of CCR2. In vitro, fractalkine is upregulated on activated SMCs via NF-κB and triggers monocyte adhesion to SMCs. After arterial injury, fractalkine is delayed expressed predominantly in endothelial cells and neointimal SMCs. Because of incomplete reendothelialization after mechanical injury, fractalkine-expressing neointimal SMCs exposed to the blood stream may enhance chronic monocyte recruitment. Indeed, monocyte adhesion was severely reduced in CX3CR1-deficient mice 5 days after wire-induced endothelial denudation, which may be responsible for the inhibition of neointima formation. However, fractalkine-dependent SMC proliferation may also contribute to neointimal hyperplasia. Of note, the CX3CR1 polymorphism V249I is associated with enhanced monocyte adhesiveness and an increased risk for restenosis after coronary stent implantation.

**Endothelial Recovery Is Mediated by Chemokines**

The CX3 chemokine keratinocyte-derived chemokine (K)/growth-regulated oncogene (GRO)-α (CXCL1) affects vascular wound healing entirely different than diet-induced atherosclerosis. Whereas KC/GRO-α promotes atherogenesis through increased monocyte recruitment most likely via CXCR2, it appears to enhance endothelial recovery. Neointimal macrophages were identified as the major source of KC in injured vessels of ApoE−/− mice and treatment with a blocking KC antibody resulted in exacerbated neointimal growth. Although the neointimal macrophage and the SMC content were unaffected, inhibition of KC clearly impaired endothelial recovery. The effect of KC on reendothelialization is most likely attributable to CXCR2, because regenerating endothelial cells in vivo express CXCR2 and KC-dependent endothelial wound healing in vitro is mediated by CXCR2. KC/GRO-α is so far the only chemokine with a vasculoprotective effect after denuding injury through enhanced vascular healing.

Reendothelialization partly depends on BM-derived recruitment of circulating endothelial progenitor cells (EPCs). Mobilization of EPCs by pharmacological interventions inhibits neointima formation by accelerated endothelial recovery. In addition to CXCR4, CXCR2 is critically involved in EPC arrest to injured carotid arteries and inhibition of CXCR2 abolished enhanced endothelial recovery after injection of EPCs. Recruitment of endogenous EPCs is not evident earlier than 2 weeks after injury, which parallels the time response of neointimal KC/GRO-α expression. Other CXCR2 ligands, such as CXCL7, CXCL8, or MIF, are expressed early in neointima formation and may participate in CXCR2-dependent EPC recruitment. The functional significance of CXCR4-mediated EPC adhesion to endothelial recovery is currently unresolved. However, treatment with a CXCR4 antagonist does not inhibit reendothelialization after endothelial denudation, suggesting a minor role in EPC recruitment.

Ex vivo activation by MCP-1 stimulates stable adhesion of BM-derived monocyte-like cells (BM-MLC) to injured arteries, which enhances reendothelialization and reduces neointima formation. In fact, blocking CCR2 after stent implantation had no effect on re-endothelialization, indicating that MCP-1-dependent activation of injected EPCs is primarily a therapeutic approach.

**Summary and Conclusions**

In summary, chemokines functionally regulate every part of arterial remodeling with a highly elaborate specialization and in cooperation of multiple chemokines (Table 2). It is also intriguing that chemokine functions vary considerably be-
tween native atherogenesis and nonatherogenic arterial remodeling. This is a major caveat for using available chemokine receptor antagonists in clinical trials. Taking this into account, certain vascular disease entities, such as restenosis after stent implantation or cardiac allograft vasculopathy, may be most approachable for therapeutic antichemokine strategies, because of the relatively defined onset and risk for disease and the possibility for site directed therapy via drug-eluting stents. In addition, other strategies to inhibit chemokine activity or expression, such as transcription factor decoys, inhibitors of signal transduction, or siRNAs, may be feasible by locally confined delivery.

Disclosures

None.

References

MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med. 2007;13:587–596.


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