Chemokines in vascular remodeling

Andreas Schober¹, Alma Zernecke²

¹Division of Cardiology, Medizinische Poliklinik, University of Munich, Munich, Germany; ²Institute of Cardiovascular Research, University Hospital Aachen, Aachen, Germany

Summary

The arterial vessel wall response to a variety of injuries consists in structural changes, which can result in luminal narrowing and aggravation of the underlying disease. This arterial remodeling is characterized by neointima formation and medial thickening, inflammatory cell recruitment and endothelial dysfunction. Chemokines and the corresponding receptors have been shown to participate at every step of the remodeling process. The monocyte chemotactic protein (MCP)-1/CC motif receptor 2 (CCR2) axis induces monocyte infiltration of the injured vessel wall and can stimulate proliferation of smooth muscle cells (SMCs) in models of restenosis, cardiac allograft vasculopathy (CAV), pulmonary hypertension, and systemic hypertension. In contrast, stromal cell-derived factor (SDF)-1α and its receptor CXC motif receptor 4 (CXCR4) are centrally involved in the neointimal recruitment of SMC progenitor cells (SPCs), presumably in response to SMC apoptosis, in restenosis and CAV. The RANTES (Regulated upon activation, normally T-cell expressed, and presumably secreted) receptors CC motif receptor 1 (CCR1) and CC motif receptor 5 (CCR5) affect intimal monocyte infiltration and neointimal growth, which could be due to the deposition of platelet-derived RANTES on activated endothelial cells. Fractalkine is expressed on neointimal SMCs and thus mediates the arrest of monocytes. Interestingly, reendothelialization of injured vessels appears to primarily depend on CXC motif ligand 1 (CXCL1). These chemokine effects form a complex network, which operates in all mechanisms of vascular remodeling. The detailed understanding of the function of the chemokine network in the remodeling process may allow specific disease intervention.

Keywords

Chemokines, leukocyte trafficking/recruitment, vascular remodeling, smooth muscle cells, stem cells

Introduction

Vascular remodeling is a broadly used term, which basically describes structural changes of the arterial wall as a response to various stimuli, such as wall shear stress, hypoxia, immunological or mechanical injuries, leading to changes in vessel size and luminal width (1–3). Depending on the causative mechanism, remodeling can result in increased (outward remodeling) or decreased arterial cross-sectional diameter (inward remodeling) with either thickening or thinning of the vessel wall (1). In many diseases, such as restenosis after percutaneous intervention, cardiac allograft vasculopathy, or pulmonary arterial hypertension, arterial remodeling plays a central role in causing a reduction in the luminal diameter (1, 4). In atherosclerosis, however, outward remodeling can occur leading to an increase of the luminal diameter and can compensate for an increased plaque load (5). In general, every layer of the arterial wall can be affected by the remodeling process, including neointimal hyperplasia, medial hypertrophy, and adventitial fibrosis.

For clinical reasons, remodeling is generally defined as any arterial size change (enlargement or contraction), independent or dependent on neointima formation (4). Neointima formation and inward remodeling constitute major clinical problems after percutaneous interventions of obstructed and stenotic arteries, which often results in significant luminal narrowing and hypoperfusion. Neointimal formation and medial thickening is mainly characterized by the accumulation of smooth muscle cells (SMCs) and extracellular matrix proteins. Disturbance of the structural and functional integrity of the endothelial cell layer can be observed in vascular remodeling diseases and appears to directly influence the accumulation of smooth muscle cells and the inflammatory response (2, 6). In addition, inflammatory cell infiltration has been described in many forms of arterial remodeling and has been proven to contribute to the progression of the disease (1, 6, 7).
Different types of chemokines and their receptors have been demonstrated to be centrally involved in all phases of arterial remodeling and may therefore provide a valuable target for disease interventions (8). In the present review the evidence for chemokine-mediated vascular remodeling by inflammatory cell recruitment, proliferation of SMCs, recruitment of vascular progenitor cells, and endothelial recovery in different diseases will be summarized (Fig. 1).

**MCP-1 (CCL2)/CCR2 axis is involved in many forms of arterial remodeling**

In experimental models of neointima formation, the role of the CC-chemokine monocyte chemotactic protein (MCP)-1/CC motif ligand 2 (CCL2) has been studied most thoroughly. After acute mechanical injury MCP-1 mRNA expression is rapidly up-regulated within 4 hours (h) with a subsequent increase of the MCP-1 protein in the arterial wall and in the circulation (9–11). Interestingly, MCP-1 expression in smooth muscle cells can be increased by platelet derived growth factor (PDGF) (9), which itself has also been shown to influence neointima formation (12), and by thrombin (13), which is generated early following arterial injury (14). This injury-induced upregulation of MCP-1 in the vessel wall, however, is only transient, and baseline levels are again observed after 3–4 days in various models of acute vascular injury (9, 10).

In the first report studying the functional role of MCP-1 in neointimal hyperplasia, treatment with high doses of neutralizing MCP-1 antibody resulted in a significant reduction in neointima formation in a rat model of carotid injury (10). This effect of MCP-1 inhibition was associated with a reduced number of neointimal smooth muscle cells, whereas the extent of leukocyte infiltration was unchanged (10). These results may imply a primary role of MCP-1 on smooth muscle cell accumulation but not inflammation in injury-induced arterial remodeling. In contrast, treatment of monkeys after balloon injury of the iliac artery with an anti-human CCR2 antibody did not reduce neointimal hyperplasia; however, neointima formation after additional stent implantation in this model could be inhibited by the CCR2 antibody treatment (15). Although macrophage infiltration was more prominent early after stent placement compared with balloon injury, the macrophage content in the vessel wall was negligible in all treatment groups after 29 days (15). In a transgenic approach, neointima formation, neointimal smooth muscle content, and SMC proliferation rates were clearly reduced after femoral artery injury in mice deficient in the MCP-1-receptor CCR2 (16). In this model of arterial injury, neointimal leukocyte infiltration was absent in CCR2−/− mice. Periarterial placement of a non-constrictive cuff around the femoral artery in mice induced neointima formation was associated with an intense, early infiltration with monocytes, and an enhanced cellular proliferation at the cuff site was detected along with a transient up-regulation of MCP-1 expression (17). In this model, neointima formation, monocyte infiltration and cell proliferation was almost completely abolished in CCR2−/− mice and in mice after gene transfer of a plasmid coding for a N-terminal deletion mutant MCP-1 (7ND), which acts as an inhibitor of MCP-1 (17). This result could be confirmed in monkeys, where the gene transfer of mutant MCP-1 resulted in reduced cuff-induced neointima formation (17). Moreover, in hypercholesterolemic rabbits gene transfer of the plasmid 7ND after balloon injury of the carotid artery appeared to reduce neointima formation primarily by inhibiting macrophage infiltration of the injured vessel (18). In addition, an effect of MCP-1 on constrictive remodeling was hypothesized, since the treatment with the deletion mutant 7ND was associated with a greater cross-sectional arterial diameter as assessed by the length of the external elastic

**Figure 1: Schematic representation of the interaction of the three components of arterial remodeling:** Inflammatory cell infiltration, smooth muscle cell (SMC) accumulation through the recruitment of SMC progenitor cells (SPC) and proliferation of SMC, and the structural or functional endothelial defect. Chemokines have been shown to regulate each of these processes in a redundant or exclusive manner: Infiltration of monocytes appears to be mediated by MCP-1, RANTES, and fractalkine, whereas SPC recruitment and re-endothelialisation are selectively directed by SDF-1α and KC/GRO-α, respectively.
Similarly, in hypercholesterolemic apolipoprotein E- (apoE) deficient mice, which were also deficient in CCR2, neointimal lesions after wire-injury of the carotid artery were significantly diminished compared with apoE+/−/CCR2+/− mice (11). This was mostly due to the marked reduction of the neointimal macrophage content, since the relative neointimal SMC content was not affected (11).

In summary, inhibition of MCP-1 quite uniformly reduced neointimal hyperplasia after arterial injury in different animal models. In injury models, however, where prominent inflammatory responses are induced, e.g. after periarterial cuff placement or endothelial denudation in hypercholesterolemic animals, MCP-1-mediated neointima formation is primarily due to an increased macrophage infiltration (Table 1). In contrast, intravascular injury in non-hyperlipidemic animals accompanied by only little inflammatory infiltrates, a reduction in neointima formation (6), is due to direct effects of MCP-1 on neointimal SMC proliferations possibly in an autocrine manner (19) (Table 1).

The mechanistic role of MCP-1 in early monocyte adhesion after wire-induced carotid artery injury has been investigated in detail in apoE−/− mice. The upregulation of MCP-1 in the injured vessel was associated with increased levels of circulating MCP-1 levels and an immobilisation of MCP-1 on platelets adherent to the injury site, but not circulating platelets (11).

### Table 1: Effect of inhibition of the MCP-1/CCR2 axis on vascular remodeling.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Effect</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHPL mouse, wire-injury, femoral artery, genetic deletion of CCR2</td>
<td>Neointima: 61.4% ↓</td>
<td>SMC proliferation ↓ Leukocytes →</td>
<td>(16)</td>
</tr>
<tr>
<td>HPL, apoE−/− mice, wire-injury, carotid artery, genetic deletion of CCR2</td>
<td>Neointima: 47% ↓</td>
<td>Macrophages ↓ SMC content → Early monocyte adhesion ↓</td>
<td>(11)</td>
</tr>
<tr>
<td>NHPL monkey, balloon injury or stent, iliac artery, Anti human CCR2 mAb</td>
<td>Balloon: → Stent: 46% ↓</td>
<td>inflammation ↓</td>
<td>(15)</td>
</tr>
<tr>
<td>NHPL mice and monkeys, cuff placement, femoral artery, gene transfer of 7ND</td>
<td>Neointima: 60% ↓</td>
<td>Macrophages ↓ SMC proliferation ↓</td>
<td>(17)</td>
</tr>
<tr>
<td>HPL, rabbit, balloon injury, carotid artery, gene transfer of 7ND</td>
<td>Neointima 40% ↓</td>
<td>Macrophages ↓ Constrictive remodeling ↓</td>
<td>(18)</td>
</tr>
<tr>
<td>NHPL, rat, balloon, carotid artery, MCP-1 Ab</td>
<td>Neointima: 55.6% ↓</td>
<td>SMC content ↓ Leukocytes →</td>
<td>(10)</td>
</tr>
<tr>
<td>HPL, mice, venous interposition in carotid arteries, gene transfer of 7ND</td>
<td>Neointima 51% ↓</td>
<td>SMC proliferation ↓</td>
<td>(30)</td>
</tr>
<tr>
<td>NHPL, mice, heterotopic cardiac transplantation, gene transfer of 7ND</td>
<td>Neointima 39% ↓</td>
<td>Leukocyte infiltration ↓</td>
<td>(32)</td>
</tr>
<tr>
<td>NHPL, rat, monocrotaline-induced PH, gene transfer of 7ND</td>
<td>Media: 29% ↓</td>
<td>Monocyte recruitment ↓</td>
<td>(34)</td>
</tr>
<tr>
<td>NHPL, rat, monocrotaline-induced PH, MCP-1 Ab</td>
<td>Medial thickening ↓</td>
<td>Macrophages ↓</td>
<td>(33)</td>
</tr>
<tr>
<td>NHPL, mice, angiotensin II-induced hypertension, genetic deletion of CCR2</td>
<td>Wall thickness: 65% ↓</td>
<td>Macrophages ↓</td>
<td>(40)</td>
</tr>
<tr>
<td>NHPL, mice, angiotensin II-induced hypertension, leukocyte-specific CCR2 deletion</td>
<td>Wall thickness ↓</td>
<td>Macrophages ↓ Proliferation ↓</td>
<td>(41)</td>
</tr>
</tbody>
</table>

NHPL=non-hyperlipidemic; HPL=hyperlipidemic; PH=pulmonary hypertension.
EPCs (25–27). Endothelial recovery after arterial balloon injury by bone marrow-derived CD14<sup>+</sup> EPCs was markedly enhanced after treatment with MCP-1 resulting in reduced neointimal lesions (28). MCP-1-stimulated EPC adhesion to endothelial cells under flow conditions was clearly increased by activating β1 integrin conformation, a mechanism that could be responsible for the MCP-1-dependent accelerated re-endothelialization in vivo (28). The elution of integrin-binding peptide cRGD from polymer-coated stents promoted the endothelial recovery and recruitment of EPCs, thereby limiting neointimal hyperplasia in a pig model of coronary stent implantation (29).

In comparison, vein graft thickening in an apoE<sup>−/−</sup> model after venous interposition in the carotid artery, where MCP-1 expression was mainly found in adhering and infiltrating leukocytes in the first week, was reduced after gene transfer of the MCP-1 antagonist 7ND, but the relative neointimal SMC and macrophage content was not affected by the inhibition of MCP-1 (30) (Table 1). Similarly, in heterotopic cardiac transplantation models, MCP-1 expression was found to be up-regulated in arteries of cardiac allografts with graft vasculopathy (31, 32) and appeared to be predominantly expressed by infiltrating monocytes (31). Intimal hyperplasia in cardiac allografts, as determined by the intima/media ratio, was reduced by neutralization of MCP-1 by 7ND gene transfer (32) (Table 1).

In pulmonary hypertension, the structural remodeling of the pulmonary vasculature in response to hypoxia is characterized by medial and adventitial thickening (2). This is induced by the recruitment of circulating progenitor cells and a hypoxia-induced inflammatory response within the vessel wall (2). In a rat model of monocrotaline-induced pulmonary hypertension, inhibition of MCP-1 by a blocking antibody or by 7ND gene transfer reduced the medial thickening in pulmonary arteries, the right ventricular pressure, and improved the mortality rate (33, 34) (Table 1). Vascular remodeling in systemic arterial hypertension, e.g. essential human hypertension, comprises endothe-loparyngic remodeling of resistance arteries and medial hypertrophy of larger arteries (3, 35). It appears to critically depend on angiotensin II-mediated vascular inflammation and arterial macrophage infiltration (36). At least in part mediated by mechanical strain and angiotensin-II, MCP-1 gene expression is increased in the aortas of hypertensive rats (37, 38). Recently, the transcription factor Ets-1 has been identified as a central regulator of angiotensin II-induced vascular inflammation and MCP-1 expression in hypertensive remodeling (39). In this model, the importance of the MCP-1/CCR2 axis was demonstrated in CCR2-deficient mice, where the aortic wall thickness and macrophage infiltration were clearly reduced in CCR2-deficient mice and in bone marrow-transferred mice with leukocyte-selective CCR2 deficiency (40, 41) (Table 1).

Vascular repair by SPCs is directed by SDF-1α (CXCL12) and CXCR4

Neointimal SMCs are not exclusively derived from resident medial SMCs, since bone marrow-derived circulating SMC progenitor cells (SPCs) can be recruited to the injured vessel wall and contribute to neointimal SMC accumulation (42). Since the migration of bone marrow-derived stem cells and their mobilization from the bone marrow is regulated by the CXC-chemokine stromal cell-derived factor (SDF)-1α (43), the role of SDF-1α in SMC-mediated neointima formation has been studied. Temporarily increased plasma levels of SDF-1α and an early and sustained upregulation of SDF-1α in SMCs following mechanical injury has been described in different models (44–49) and has been shown to mediate the mobilization of circulating SPCs (44). Blocking SDF-1α in apoE<sup>−/−</sup> mice after wire-injury resulted in a significant reduction of the neointimal area by inhibiting the accumulation of bone marrow-derived SPCs in the neointima, and thus diminished the neointimal SMC content (44, 45). The effect of SDF-1α on SPC recruitment is dependent on the expression of the SDF-1α receptor CXCR4 (CXCR4<sup>+</sup>) in bone marrow cells, since both neointimal hyperplasia and SMC content is diminished in apoE<sup>−/−</sup> mice after bone marrow reconstitution with fetal hematopoietic stem cells from CXCR4<sup>+</sup> mice (45). Interestingly, the infiltration of macrophages into the neointimal tissue was not diminished by blocking the SDF-1α/CXCR4 axis (44, 45). Findings that the lentiviral gene transfer of an antagonistic mutant SDF-1α locally to the carotid artery reduces neointima formation, suggests a critical role of local SDF-1α expression in the injured vessel herein (45).

SDF-1α can bind surface-adherent platelets and thus appears to significantly increase the P-selectin-dependent arrest of progenitor cells to the injured vessel. Furthermore, platelets have been shown to serve as a source for SDF-1α, and its release was implicated in progenitor cell recruitment after arterial thrombosis (50). The PDGF-β receptor (PDGFR-β) is expressed on the great majority of lin/sca-1<sup>−/−</sup> SPCs attracted by SDF-1α. Since the PDGF-β receptor has been found on fetal SMCs (51) and mediates the differentiation of bone marrow progenitor cells to SMCs (52, 53), expression of PDGFR-β on lin/sca-1<sup>−/−</sup> peripheral cells may specifically characterize the SPC subpopulation involved in SDF-1α-dependent neointima formation. According to this finding, SDF-1α induced the mobilization and recruitment of c-kit<sup>+</sup> and c-kit<sup>+</sup> SPCs, with a majority of neointimal SMCs originating from non-hematopoietic c-kit<sup>+</sup>/lin/sca-1<sup>−/−</sup> stem cells expressing PDGFR-β (45).

The injury-induced SDF-1α expression in arterial tissue and the degree of neointimal SPC recruitment appears to critically depend on the type of injury. Compared with carotid ligation and periarterial cuff placement, wire-induced injury produced the strongest SDF-1α expression (46) and was shown to correlate with the degree of apoptosis in medial cells. In vitro, the scratch-induced apoptosis of SMCs, presumably by the release of apoptotic bodies, resulted in an enhanced SDF-1α expression in uninjured SMCs (45).

In a carotid ligation model of neointima formation, SDF-1α expression was further increased in endothelial nitric oxide synthase-deficient mice and correlated with progenitor cell mobilization and adventitial recruitment (49). These findings indicate that the intact endothelium may confine the vascular remodeling after injury by regulating SDF-1α expression.

Bone marrow-derived vascular cell progenitors also contribute to neointimal growth and neointimal SMC content in graft vasculopathy (42, 54). In a murine aortic transplantation model, SDF-1α is upregulated in aortic allografts in the adventi-
tia and subsequently in the media and neointima (55). The increased SDF-1α expression was associated with the recruitment of recipient-derived CXC4-positive cells in aortic allografts, which can differentiate into SMCs in vitro. Treatment with a neutralizing SDF-1α antibody effectively inhibited neointima formation in aortic allografts, and reduced the number of CXC4-positive cells in the circulation and in the neointima (55). These results suggest that SDF-1α promotes neointima formation in graft vasculopathy and after mechanical injury similarly by mobilization and recruitment of SPCs.

Furthermore, the comparison of transplanted human hearts with and without signs of peritransplant ischemic injury demonstrated an increased SDF-1α expression in myocardial tissue after ischemic injury (56). This was associated with the recruitment of recipient progenitor cells exclusively to the vasculature of hearts with ischemic injury, representing vascular SMCs and endothelial cells (56). These findings may explain the increased incidence of transplant vasculopathy in ischemically damaged transplanted hearts by the enhanced SDF-1α-dependent recruitment of circulating SPCs and EPCs.

The transcriptional regulation of SDF-1α expression has recently been described under hypoxic conditions. Activation of the transcription factor hypoxia-inducible factor (HIF)-1α clearly induces SDF-1α expression in endothelial cells by binding to HIF-1α-specific binding sites in the SDF-1α promoter (57). Interestingly, non-hypoxic transcriptional and translational up-regulation of HIF-1α has been described in SMCs (58–60). We found recently, that vascular injury induces HIF-1α in SMCs and that the inhibition of HIF-1α transcription reduces neointimal SDF-1α expression and neointimal growth (Karshovska et al. 2007, submitted). These results suggest a prominent role of HIF-1α as an upstream regulator of SDF-1α expression after vascular injury.

These findings imply that the expression of SDF-1α after injury critically regulates vascular repair and remodeling through the recruitment of SPCs, a mechanism activated by apoptosis and an increased demand for SMC replacement.

**RANTES (CCL5) and its receptors CCR1 and CCR5 in vascular remodeling**

The CC-chemokine RANTES (Regulated upon activation, normally T-expressed, and presumably secreted) is stored in platelets and can be released upon platelet activation (61). Under flow conditions, platelet-derived RANTES can be immobilised on activated endothelial cells in the presence of platelet P-selectin, and thereby supports monocyte adhesion and atherogenesis (62–64). RANTES has also been detected on endothelial cells covering neointimal lesions (63) and treatment of apoE-/- mice with Met-RANTES, an antagonist for the RANTES receptors CC motif receptor 1 (CCR1) and CC motif receptor 5 (CCR5), significantly reduced neointima formation and neointimal macrophage infiltration (64). This effect might be primarily due to an interaction of RANTES with CCR5, since CCR5-deficient but not CCR1-deficient apoE-/- mice display reduced neointimal lesions (65). In addition to a reduction in the neointimal macrophage content, CCR5 deficiency entailed an up-regulation of the anti-inflammatory cytokine interleukin 10 (IL-10) in SMCs and was associated with a decrease in CD3+ T-lymphocytes (65). Conversely, proinflammatory interferon-γ was increased in the neointima of CCR1-/- mice, and its blockade unmasked a reduction in macrophage recruitment (65).

In addition to platelet P-selectin, junctional adhesion molecule (JAM)-A has been implicated in endothelial RANTES deposition. JAM-A belongs to the IgG superfamily and is a component of endothelial and epithelial tight junctions (66). Trans-endothelial migration of leukocytes, including monocytes and neutrophils, is tightly regulated by endothelial JAM-A which interacts with the β3 integrin lymphocyte function-associated antigen-1 on leukocytes (66–68). JAM-A is upregulated in endothelial cells of hyperlipidemic apoE-deficient mice and mediates atherogenic leukocyte recruitment (69). Furthermore, JAM-A is also expressed on platelets and homophilic interaction of platelet and endothelial JAM-A is important for platelet adhesion to cytokine-stimulated endothelial cells (70). Interestingly, the luminal expression of RANTES after wire-induced carotid injury in JAM-A+/−/apoE− mice was clearly reduced, which explains the impaired neointimal recruitment of monocytes and diminished neointimal growth in JAM-A deficient mice (71). The reduced luminal RANTES expression appears to be due to a defect in the endothelial deposition of platelet-derived RANTES in the absence of JAM-A (71).

In cardiac allograft vasculopathy (CAV), enhanced RANTES expression has been described in mononuclear cells infiltrating the coronary arteries, in endothelial cells of microvessels, and in intimal SMCs (72, 73). Additionally, in a rat model of cardiac and aortic transplantation RANTES and its receptor CCR5, along with interferon-γ-inducible protein (IP) 10-CXC motif receptor 3 (CXCR3) and MCP-1-CCR2, appear to be selectively up-regulated in the arterial wall during neointima formation (74). A clear functional role of the RANTES receptors CCR1 and CCR5 in CAV has been established in a mouse model using the RANTES receptor antagonist Met-RANTES (75), which reduced the neointimal growth and the infiltration of the vessel wall with mononuclear cells (75). It is reasonable to suggest that this inhibition of CAV by blocking CCR1 and CCR5 is at least in part responsible for the enhanced allograft survival described in CCR5−/− (76) and in CCR1−/− mice (77), after pharmacological inhibition of CCR1 (78), and by gene transfer of RANTES antagonists (79, 80).

**Fractalkine/CX3CR1 in restenosis and pulmonary hypertension**

Although the CX3C chemokine fractalkine (CX3C motif ligand 1, CX3CL1) and its receptor CX3C motif receptor 1 (CX3CR1) have been shown to play a direct role in atherogenesis by the inhibition of monocyte recruitment (81–83), the contribution of the CX3CL1/CX3CR1 axis to neointima formation after vascular injury is less clear. In vitro, fractalkine is expressed on neointimal SMCs and thereby effectively mediates monocyte adhesion (48). Since complete re-endothelialization of the injured vessel may take several weeks in animal models (84) and severe damage to the vessel wall may even result in a permanent replacement of endothelial cells by activated SMCs (85, 86),
neointimal SMCs become exposed to the blood stream and may enhance chronic monocyte recruitment to neointimal lesions via fractalkine expressed on SMCs. This notion is supported by results obtained from cytokine-stimulated vascular SMCs, where fractalkine is upregulated by nuclear factor (NF)-κB activation, induces SMC proliferation (87), and mediates monocyte adhesion to SMCs (88). In a murine model of femoral artery denudation, fractalkine was upregulated in intimal SMCs and endothelial cells (89). In CX3CR1-deficient mice, neointimal hyperplasia was significantly reduced compared with wild-type (89). This was associated with a decreased infiltration of monocytes to the injured vessel and a diminished rate of SMC proliferation (89). Interestingly, the CX3CR1 polymorphism V249I in patients is associated with enhanced monocyte adhesiveness (90) and an increased risk for restenosis after coronary stent implantation (91).

In contrast to mechanical vascular injury, fractalkine was present on perivascular inflammatory cells and CX3CR on medial smooth muscle cells in a rat model of pulmonary hypertension (92). However, the functional significance of this finding for vascular remodeling in pulmonary hypertension remains to be determined.

### CXCL1 (KC/GRO-α) and CXCR2 enhance endothelial recovery after arterial injury

In native atherosclerosis monocytic CXC motif receptor 2 (CXCR2) expression appears to be critical for the intimal accumulation of monocytes (93), most likely due to the arrest function of keratinocyte-derived chemokine (KC)/growth-regulated oncogene (GRO)-α on activated endothelial cells (94, 95). However, inhibition of KC by a blocking antibody after carotid wire injury in apoE−/− mice resulted in increased neointimal area and impaired endothelial recovery (96). In contrast, the neointimal macrophage and SMC content were unaffected by the KC antibody treatment. Intraluminal macrophages accumulating two weeks after injury were identified as the major source of KC in the injured vessel (96). Since regenerating endothelial cells express CXCR2 in vivo and KC induces endothelial wound healing via CXCR2 in vitro (96), a central role of KC and CXCR2 in endothelial recovery can be presumed. Thus, neointimal macrophages by secreting KC may induce endothelial recovery, which may be vasculo-protective (97). Therefore, chemokines are not only involved in the initiation of vascular remodeling, but may also mediate vascular healing by endothelial recovery.

Transplantation of ex vivo expanded EPCs has been shown to accelerate endothelial recovery and thereby reduce neointima formation after vascular injury (27). Whether KC/GRO-α and its receptor CXCR2 enhance regeneration of the endothelial cell layer by the recruitment of circulating EPCs has been recently studied. In perfusion studies of murine carotid arteries, firm arrest of CXCR2-expressing EPCs 24 h after injury was inhibited by a CXCR2 antibody (98). Therapeutic infusion of human KDR′/CXCR2′ EPCs into athymic nude mice after vascular injury resulted in an enhanced endothelial recovery, which was abolished in EPCs pretreated with CXCR2 antibody (98). In contrast to the CXCR4-mediated SPC mobilization and recruitment, homing of EPCs after endothelial injury critically depends on CXCR2. KC/GRO-α has been not detected in the carotid artery 24 h after injury (96), therefore other CXCR2 ligands, such as CXCL7 and CXCL8, which have been found at the injury site after 24 h, may be involved in EPC-mediated endothelial recovery (98).

### Conclusions and perspectives

In summary, chemokines functionally regulate different phases of arterial remodeling with a highly elaborate specialization and in cooperation of multiple chemokines. It is also intriguing that chemokine functions vary considerably between native atherogenesis and non-atherogenic 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 graft vasculopathy, may be most approachable for therapeutic anti-chemokine 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.


