Potential cell-specific functions of CXCR4 in atherosclerosis

Christian Weber1-3; Yvonne Döring1; Heidi Noels4

1Institute for Cardiovascular Prevention, Ludwig-Maximilians-University, Munich, Germany; 2German Centre for Cardiovascular Research, partner site Munich Heart Alliance, Germany; 3Cardiovascular Research Institute Maastricht, University of Maastricht, the Netherlands; 4Institute for Molecular Cardiovascular Research, RWTH Aachen University, Aachen, Germany

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Summary
The chemokine CXCL12 and its receptor CXCR4 form an important axis contributing to cellular functions in homeostasis and disease. In addition, the atypical CXCL12 receptor CXCR7 may shape the availability and function of CXCL12. Further to their role through progenitor cell mobilization, CXCL12 and CXCR4 may affect native atherogenesis by modifying atherosclerosis-relevant cellular functions. This short review intends to provide a concise summary of current knowledge with regards to cell-specific functions of CXCL12 and its receptors CXCR4 and CXCR7 with potential implications for the initiation and progression of atherosclerosis.

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Zusammenfassung

Correspondence to:
Prof. Dr. med. Christian Weber
Institute for Cardiovascular Prevention (IPEK)
Ludwig-Maximilians-University Munich
Pettenkoferstraße 9, 80336 Munich, Germany
E-mail: chweber@med.lmu.de

Chemokines are a structurally related family of chemotactic cytokines that are classified in subgroups based on the position of the N-terminal cysteine residues (CC, CXC, CX3C, C). Expressed by not only activated endothelial cells and smooth muscle cells but also emigrated leukocytes, chemokines directly recruit leukocytes to sites of inflammation. Recent evidence has emerged to demonstrate that chemokines also exert functions beyond cell recruitment, e.g. controlling cell homeostasis and activation (1). For instance, the chemokine CXCL12 is crucial in controlling mobilization of haematopoietic stem cells and the homeostasis of many cell types. Expression of the CXCL12 (also known as stromal cell-derived factor 1) receptor CXCR4 has been described on many cell types including macrophages, neutrophils (2), T-cells (3), B-cells (4), mature endothelial cells (ECs) (5), and vascular smooth muscle cells (VSMCs) (6, 7).

While all of these cell types play distinct roles in the pathophysiology of atherosclerosis (8), little is known about the precise role of CXCR4 during individual cellular responses in this context (Fig. 1).

Macrophages, neutrophils

CXCL12/CXCR4 signalling has been linked to enhanced macrophagocytosis in leukocytes (9), suggesting that a lack of CXCR4 may influence (modified) lipid accumulation in macrophages and other lesional cells. In contrast, a recent study found CXCL12 to induce phagocytosis and the uptake of acetylated LDL in THP1-derived macrophages through binding CXCR7 but not CXCR4 (10). Accordingly, the CXCR7 agonist CCX771 was shown to increase the uptake of very low-density LDL (VLDL) in adipocytes and treatment of Apoe−/− mice with CCX771 reduced the levels of circulating VLDL and decreased atherosclerosis (11).

Whether similar mechanisms can be identified in cell types other than macrophages remains to be determined, as are the exact mechanisms underlying CXCR7-mediated uptake of (modified) lipids. In contrast to human monocytes, expression of CXCR4 in mouse is higher expressed on non-classical compared to classical monocytes but this may not imply functional differences in individual mouse and human monocyte subsets (12). Treatment of mice with the CXCR4 antagonist AMD3100 induced cell egress...
from the bone marrow (13), which is in line with findings of increased leukocytosis, mostly neutrophil mobilization, and enhanced lesion formation in Apoe−/− receiving a cholesterol-rich diet for 12 weeks while treated with AMD3100. Interestingly, monocyte numbers were only moderately enhanced in these mice and lesion growth was mainly attributable to increased plaque neutrophils and enhanced apoptosis (14). Subsequently emerging evidence underscored the role of neutrophils in atherogenesis (15, 16) and it was recognized that the CXCL12/CXCR4 axis maintains neutrophil homeostasis by regulation of neutrophil release from the bone marrow in a cell-autonomous fashion (17). Furthermore, senescent neutrophils in the periphery expressing high levels of CXCR4 can home back to the bone marrow to be cleared in a rhythmic fashion (18, 19). Accordingly, neutrophil mobilization by AMD3100-mediated CXCR4 inhibition has also been attributed to arise from lung demargination and blockade of neutrophil homing to the bone marrow (20), and consequently the increase of atherosclerosis by CXCR4 blockade may be partially due to perturbing neutrophil apoptosis and senescence. Also, CXCR4 blockade through expression of CXCR4 degrakine in bone marrow cells was shown to enhance atherosclerosis by affecting neutrophil function, as reflected in a hyperactivated state, increased adhesion capacity and reduced apoptosis (21).

**Lymphocytes, platelets**

In addition to CXCL12/CXCR4 mediating lymphocyte recruitment, CXCR4 is able to mediate MIF-induced chemotaxis of B-cells and T-cells, and T-cell arrest in vitro (22, 23). Blockade of MIF in Apoe−/− mice on high-fat diet indeed resulted in the formation of smaller atherosclerotic lesions displaying reduced macrophage and T-cell content, supporting a role for MIF in T-cell chemotaxis in the context of atherosclerosis in vivo (22).

CXCL12 has been reported to be a weak platelet agonist amplifying platelet activation, adhesion and chemokine release triggered by low dose agonists such as ADP, thrombin, or arterial flow (24, 25). Furthermore, CXCL12 gradients could induce platelet migration and transmigration in vitro involving PI3K signalling (26). In addition, CXCL12 can trigger CXCR4 internalization and cyclophilin A-dependent CXCR7 externalization on platelets, resulting in prolonged platelet survival. Mice lacking the cytosolic chaperone cyclophilin A showed less CXCL12-induced rescue of platelets from activation-induced apoptosis through CXCR7 engagement (27). Likewise, MIF can limit activation-induced apoptosis of platelets via CXCR7-dependent Akt signalling, thereby reducing the thrombotic potential (28). Hence, differential regulation of CXCR4/CXCR7 surface expression on platelets upon ligand exposure at sites of platelet activation or accumulation may orchestrate platelet survival. In turn, the vascular functions mediated by platelet CXCR4 remain to be elucidated in vivo.

**Endothelial cells**

Expression of CXCR4 on various types of vascular ECs has been widely reported (29), however, ECs represent a very heterogeneous population, with ECs from different anatomic sites differing in protein expression, localization and function (30), particularly when venous and arterial ECs are concerned (31). In human carotid artery specimens, CXCR4 was abundantly expressed by lesional ECs and only marginally in minimally diseased endothelium (32, 33). Similarly, Gupta et al. showed CXCR4 mRNA expression in human coronary artery ECs, although it is not clear whether these ECs were derived from inflamed or steady-state endothelium (5). CXCR4 was also found to be expressed in bovine aortic ECs, where it was redistributed from a cytoplasmic granular pattern to the surface in migrating cells (34); in human aortic ECs, where it was upregulated by stimulation with VEGF or bFGF (35); and in mouse aortic endothelium, where it may be down-regulated by atheroprotective shear flow, similarly as observed in HUVECs where low shear stress led to increased apoptosis (33, 36). As angiogenesis may contribute to plaque vulnerability and hemorrhage, a potential role of CXCR4/CXCL12 therein is important to consider. Interestingly, blocking of TLR2 appears to promote the angiogenic capacity of ECs, by inducing ERK1/2 and Akt1 signalling through CXCR4, by modulating a TLR2-CXCR4 association (37).

Recently, CXCL12 has been identified as a suppressor of endothelial permeability,
promoting endothelial barrier integrity through CXCR4 but not CXCR7 (38). Specifically, CXCL12 stimulates the CXCR4/PI3K/Rac1 signaling pathway for subsequent cytoskeletal rearrangement. As another potential mechanism for protective effects, CXCL12-CXCR4 has been shown to induce eNOS phosphorylation and activity, thereby increasing endothelial NO availability (39). Furthermore, apoptotic microparticles derived from distressed ECs conferred microRNA-126 to resident ECs to unleash autocrine CXCL12 induction through CXCR4 by repressing its inhibitor RGS16, triggering angiogenic cell recruitment and atheroprotective endothelial regeneration (40). A role for CXCR4 signaling in endothelial recovery was furthermore supported by the observation that endothelial-specific knock-out of CXCR4 reduced reendothelialization after endothelial denudation, which was associated with reduced mobilization of endothelial progenitor cells and enhanced neointima formation (41).

Also, the alternative CXCR4 ligand MIF has been reported to mediate EC migration and tube formation, and to drive angiogenesis in matrigel plugs in vivo (42). Furthermore, MIF stimulation of ECs induces the secretion of CCL2 and the expression of the adhesion molecule ICAM-1 (43, 44), which could contribute to atherogenesis by mediating leukocyte recruitment and their adhesion on the endothelium. However, it remains unclear to which extent these observations depend on the chemokine receptor CXCR4, as ECs also express the MIF receptors CXCR2 and CD74, the latter under inflammatory conditions (45).

VSMCs

Vascular smooth muscle cells (VSMCs) are highly specialized cells controlling contraction and regulation of blood vessel diameter, blood pressure and blood flow. Moreover, VSMCs play a critical role in secretion of extracellular matrix components, which shape the mechanical properties of mature blood vessels. Differentiated VSMCs in adult blood vessels proliferate at very low rates and retain high plasticity, enabling changes in phenotype, referred to as phenotypic switching (41), which is considered an important pathophysiological mechanism in atherosclerosis (42). A functional CXCR4 expression in SMCs has been implied by findings that addition of CXCL12 or HIV envelope proteins specifically binding CXCR4 did induce tissue factor activity in human aortic SMCs (43). Subsequent reports confirmed CXCR4 protein expression on human vein SMCs (44), CXCR4 expression (RNA, protein) on mouse medial SMCs (6), rat (7, 45) and human aortic SMCs (Weber et al., unpublished data).
Besides switching to a synthetic secretory phenotype and their presumed migration from the medial to the intimal arterial wall giving rise to atherosclerosis (42), intimal SMCs can also stabilize atherosclerotic lesions by fibrous cap formation. In a rat model of diabetes, high glucose levels were shown to trigger activation, proliferation and enhanced chemotaxis of VSMCs via the CXCL12/CXCR4 axis (7). It was described that CXCL12 stimulates pro-MMP-2 expression in human aortic SMCs via CXCR4 in association with the epidermal growth factor receptor in vitro, suggesting that CXCR4 expands its signaling repertoire by cross-talking with other receptors.

Notably, oxLDL as another proatherogenic trigger was found to induce rat aortic SMC proliferation. This effect could even be enhanced by addition of CXCL12 concomitant with diminished SMC apoptosis, which may be
- beneficial through plaque stabilization
- or
detrimental due to intimal hyperplasia (46).

Likewise, CXCL12/CXCR4 signalling might be crucial in vein graft atherosclerosis and contribute to SMC-mediated vein graft neointimal hyperplasia in mice, in association with CXCR4-mediated recruitment of inflammatory (progenitor) cells (47), similar as seen for wire-induced neointima formation (48). Systemic treatment with CXCL12/4 promotes a more stable atherosclerotic lesion phenotype and enhances the accumulation of smooth muscle progenitor cells in these lesions without promoting atherosclerosis, representing a promising approach to treat unstable atherosclerosis (49).

Conflict of interest
The authors declare that they have no conflict of interest.

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