Platelet Chemokines in Vascular Disease

Christian A. Gleissner, Philipp von Hundelshausen, Klaus Ley

Abstract—Platelets are a rich source of different chemokines and express chemokine receptors. CXCL4 is highly abundant in platelets and involved in promoting monocyte arrest from rolling and monocyte differentiation to macrophages. CXCL4 can also associate with CCL5 and amplify its effect on monocytes. The megakaryocyte CXCL7 gene product is proteolytically cleaved into the strong neutrophil chemoattractant, NAP-2, which has also been implicated in repair cell homing to vascular lesions. Platelet adhesion can induce release of CCL2 and CXCL8 from endothelial cells. Conversely, the chemokines CCL17, CCL22, and CXCL12 made by other cells amplify platelet activation. Platelet chemokines enhance recruitment of various hematopoietic cells to the vascular wall, fostering processes such as neointima formation, atherosclerosis, and thrombosis, but also vessel repair and regeneration after vascular injury. (Arterioscler Thromb Vasc Biol. 2008;28:1920-1927)

Key Words: angiogenesis ■ atherosclerosis ■ platelets ■ thrombosis ■ chemokines

The response-to-injury hypothesis of atherosclerosis emphasized a central role of endothelial denudation and was later replaced by the view that atherosclerosis is an inflammatory disease. The complex composition of the cellular infiltrate in the arterial wall clearly implicates the immune system in atherogenesis. Numerous findings reflecting the inflammatory and immune modulatory capacity of platelets have increased our knowledge about their function in vascular disease. A role for platelets and platelet-derived factors in atherosclerosis beyond their role in hemostasis has been suggested for a long time. Especially, platelet-derived chemokines have been demonstrated to be important in the pathogenesis of atherosclerotic disease including neointima formation and thrombosis.

Platelets are anucleate cellular fragments derived from megakaryocytes in the bone marrow that play an important role in hemostasis. Among various soluble factors, chemokines constitute a significant portion of α-granule contents which are released within seconds after platelet activation. Chemokines are chemotactic cytokines that, depending on the position of cysteine residues within their structure, can be classified into different families (CXCL, CCL, CX3CL1, and XCL1). Most chemokines signal through 7-transmembrane receptors (GPCRs), coupled to G-type proteins that can be inhibited by pertussis toxin (PTX) derived from Bordetella pertussis. The classification of chemokines into the CC- and CXC-type reflects the dogma that CC-chemokines only bind to CC-chemokine receptors and CXC chemokines bind only to CXC chemokine receptors. Within each family, binding of chemokines to chemokine receptors is highly promiscuous. Thus, a given chemokine receptor may bind several different chemokines and vice versa.

Platelets may be activated by chemokines, induce chemokine expression in other cell types, and release chemokines quickly upon their own activation. In the latter group, CXCL4 (platelet factor 4, PF4) and the chemokine CXCL7, which is processed from platelet basic protein through connective tissue-activating peptide-III and β-thromboglobulin to its active form neutrophil-activating peptide-2, are the most abundant. Both CXCL4 and CXCL7 are found in high micromolar concentrations in the α-granule releasate. A number of other chemokines have been identified in platelets, even though platelets may not represent their major source. This review discusses the interactions between platelets and chemokines in the context of vascular disease, focusing on chemokines that activate platelets, platelet-induced chemokine activation or secretion by other cells, secretion and...
deposition of chemokines by activated platelets, and platelet chemokines that induce cell differentiation.

Chemokines That Activate Platelets
Platelets express a number of chemokine receptors including CCR1, CCR3, CCR4, CXCR4, and CX3CR1. Accordingly, several chemokines have been demonstrated to activate platelets inducing calcium signaling, aggregation, and release of biochemically active mediators. CCL17 (thymus and activation-regulated chemokine, TARC), CCL22 (macrophage-derived chemokine, MDC), and CXCL12 (stromal cell-derived factor-1α, SDF-1α) have been shown to activate platelets and amplify their aggregation via CCR4 and CXCR4, respectively. P-selectin induction was also demonstrated in platelets after stimulation with recombinant CX3CL1 via CX3CR1, an effect which was inhibited by PTX. Circumstantial evidence suggests that these chemokine effects may be important in atherosclerotic disease: CXCL12 and CX3CL1 are expressed in atherosclerotic lesions and elevated serum levels of CCL18 (pulmonary and activation-regulated, PARC) and regulated on activation, and normal T cell expressed and secreted (RANTES, CCL5) have been found in patients with unstable angina.

Platelets Induce Activation and Chemokine Secretion by Other Cells
Activated platelets have been described to promote cell activation and chemokine expression in several cell types relevant in atherogenesis, including endothelial cells, monocytes, and smooth muscle cells. In endothelial cells, platelets may induce CCL2 (monocyte chemotactic protein [MCP] 1) and CXCL8 (interleukin [IL] 8) as well as the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) leading to subsequent recruitment of leukocytes. This process is dependent on the cell surface receptor CD40 interacting with platelet CD40 ligand (CD40L or CD154). The potential in vivo relevance of this mechanism is underlined by the finding that disrupting CD40 signaling significantly reduces atherosclerotic lesions in the two most commonly used mouse models of atherosclerosis, the LDL receptor–deficient mice (Ldlr−/−) and the apolipoprotein E knockout mice (Apoe−/−). Ldlr−/− mice treated with anti-CD40L antibodies and Apoe−/− mice deficient for CD40L are both protected from atherosclerosis.

CXCL7
The most abundant platelet chemokine is CXCL7. In contrast to other chemokines, there are several known molecular variants of CXCL7 including platelet basic protein (PBP), connective tissue-activating peptide III (CTAP-III), β-thromboglobulin (β-TG), and neutrophil-activating peptide-2 (NAP-2) (see Figure). These CXCL7 variants are proteolytically derived from a precursor molecule (preplatelet basic protein [pre-PBP]) encoded by the CXCL7 gene. Proteolytic processing of CXCL7 can be accomplished by neutrophil-derived cathepsin G and is inhibited by interaction of CXCL7 with CXCR4. The relative proportions of precursor and truncated forms of CXCL7 change during maturation from high levels of pre-PBP in megakaryocytes to high levels of CTAP-III released on platelet activation. The only CXCL7 variant actually displaying chemotactic activity is NAP-2. The other variants may be considered precursors of NAP-2 even though they also have other specific biological activities as discussed below.

Activated Platelets Present, Secrete, and Deposit Chemokines and Thereby Induce Recruitment of Other Cells
It is not known whether CXCL7 is immobilized on the endothelium as has been described for CCL5 and CXCL4.41,42 There is little direct evidence for a proatherogenic role for platelet-derived CXCL7. Recently, CXCL7 was found in mouse carotid arteries after wire injury.43,44 CXCL7 has been shown to induce adhesion of endothelial progenitor cells (EPCs) under flow and after arterial injury in vivo through its receptor CXCR2.43 Thus, platelet-derived CXCL7 may not only be important for creating an inflammatory environment, but also for regenerating vascular integrity after injury.

CCL5
CCL5 is secreted by lymphocytes, but platelets also represent an important source.45 CCL5 binds to CCR1, CCR3, and CCR54 and induces adhesion and transmigration of monocytes and T lymphocytes in a manner that depends on integrins and adhesion molecules like ICAM-1 and VCAM-1.46,47 In mice, platelets have been demonstrated to deliver CCL5 (and CXCL4) to the monocyte surface and the endothelium resulting in increased leukocyte adhesion to the vascular wall.41 Furthermore, it has been shown that the combination of CXCL4 and CCL5 promotes monocyte adhesion to activated HUVECs under flow in a greater number than each of the chemokines alone.42 It is thought that CXCL4 may form heterodimers with CCL5 and thereby promote CCL5 binding to monocytes. Dimerization of CXCL4 with CCL5 may also induce heterodimerization of chemokine receptors, which might modulate intracellular signaling, but direct evidence for this process is lacking.

CXCL4 and CXCL4L1
CXCL4 is stored in the α-granules of the platelets and released into the plasma in concentrations ranging from 0.4 to 1.9 μmol/L.15 It is the only platelet-derived CXCL chemo-kine lacking the ELR amino acid sequence at its amino terminus, which is required for binding to CXCR1 and CXCR2.4 CXCL4 binds a 200-kDa chondroitin sulfate proteoglycan on the surface of human neutrophils, which is most likely attached to an unidentified core protein.48 CXCL4 binding was abolished when cells were pretreated with chondroitinase A. In human microvascular endothelial cells, CXCL4 has been shown to bind to CXCR3B, a splice variant of CXCR3, but not to CXCR3A, which is the receptor for the T helper-1 chemokines CXCL9, 10 and 11.49 CXCL4 signaling through CXCR3B was not PTX-sensitive, suggesting a signaling
A recent report demonstrated that chemotactic activity of CXCL4 toward human T lymphocytes is mediated by both CXCR3 splice variants (CXCR3B/CXCR3A) in a PTX-sensitive manner.50 Human neutrophil adhesion in response to CXCL4 is blocked after treatment with a src kinase inhibitor.51 Furthermore, blocking studies demonstrate a functional role for Syk, RAS, and JNK in the adhesion process. 51 Neutrophil exocytosis as determined by lactoferrin release induced by a combination of tumor necrosis factor (TNF) and CXCL4 required p38 MAPK and PI3K.50 Taken together, these findings suggest that CXCL4 triggers more than one signaling pathway.

Chemotactic activity of CXCL4 has been controversial. Early reports suggested that CXCL4 exerts chemotactic activity toward neutrophils and monocytes.52 However, these findings were not confirmed and may have been caused by contamination with other chemokines like CCL5.53–55

A role for CXCL4 in atherosclerosis had been suggested a quarter century ago.56

### Table. Synopsis of Platelet Chemokine Interactions

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Receptor(s)</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL17</td>
<td>CCR4</td>
<td>Platelet activation</td>
<td>18, 19</td>
</tr>
<tr>
<td>CCL22</td>
<td>CCR4</td>
<td>Platelet activation (P-selectin expression, adhesion)</td>
<td>18, 19</td>
</tr>
<tr>
<td>CXCL12</td>
<td>CXCR4</td>
<td>Platelet activation (P-selectin expression, adhesion)</td>
<td>19</td>
</tr>
<tr>
<td>CXCL3C1</td>
<td>CX3CR1</td>
<td>Platelet activation (P-selectin expression)</td>
<td>17</td>
</tr>
</tbody>
</table>

### Platelets that induce activation and chemokine secretion by other cells

- **CXCL1**: CXCR2
  - Oxidative stress, eNOS downregulation in EC
  - EC activation (E-selectin expression)
  - Monocyte activation (oxidative burst, induction of CCL3, CCL4, and CXCL8, cytotoxicity for EC)

- **CXCL4**: CXCR3B
  - Adhesion of monocytes to EC (in conjunction with CCL5)
  - Neutrophil adhesion
  - Neutrophil exocytosis (in conjunction with TNFα)
  - Lack results in reduced size of atherosclerotic lesions in Apoe<sup>−/−</sup> mice

### Platelet chemokines that induce differentiation of other cells

- **CXCL4**: 200 kDa chondroitin sulfate proteoglycan, CXCR3<sup>B</sup> (<sup>CXCR3A</sup>)
  - Monocyte differentiation to macrophages, specialized antigen-presenting cells, or foam cells
  - Inhibition of EC proliferation and angiogenesis
  - Inhibition of T cell proliferation and deviation towards Th2 phenotype
  - Inhibition of megakaryopoiesis

- **CXCL7**: CXCR1, CXCR2
  - Synthesis of matrix components by fibroblasts
  - Differentiation of CD34+ progenitor cells to EC

- **CXCL12**: CXCR4
  - Differentiation of CD34+ progenitor cells to EC

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mechanism different from that of other chemokine receptors. A recent report demonstrated that chemotactic activity of CXCL4 toward human T lymphocytes is mediated by both CXCR3 splice variants (CXCR3B>CXCR3A) in a PTX-sensitive manner.50 Human neutrophil adhesion in response to CXCL4 is blocked after treatment with a src kinase inhibitor.51 Furthermore, blocking studies demonstrate a functional role for Syk, RAS, and JNK in the adhesion process.51 Neutrophil exocytosis as determined by lactoferrin release induced by a combination of tumor necrosis factor (TNF)α and CXCL4 required p38 MAPK and PI3K.50 Taken together, these findings suggest that CXCL4 triggers more than one signaling pathway.

Chemotactic activity of CXCL4 has been controversial. Early reports suggested that CXCL4 exerts chemotactic activity toward neutrophils and monocytes.52 However, these findings were not confirmed and may have been caused by contamination with other chemokines like CCL5.53–55

A role for CXCL4 in atherosclerosis had been suggested a quarter century ago.56 Both platelets and CXCL4 are present
in atherosclerotic lesions and correlate with severity of atherosclerotic lesions and clinical parameters. Recently, a causal role of CXCL4 in atherogenesis was suggested by reduced lesion sizes in CXCL4-deficient Apoe<sup>−/−</sup> mice. As discussed above, this may be attributable to reduced platelet-dependent deposition of CXCL4 and CCL5 on endothelial cells and thus reduced monocyte recruitment.26

CXCL4L1 (initially called PF4alt), the transcript of a gene highly homologous to CXCL4, has also been demonstrated to be present in human platelets. CXCL4L1 differs from CXCL4 by substitution of three amino acids in the C-terminal alpha helix. Both CXCL1 and CXCL4L1 have similar levels of homology to the rodent gene for CX4, and it seems likely that the gene duplicated after the divergence of the two species. The functional characteristics of CXCL4L1 differ from those of CXCL4; most strikingly, CXCL4L1 has been demonstrated to be a much stronger inhibitor of endothelial cell chemotaxis than CXCL4. So far, no receptor for CXCL4L1 has been identified.

**CXCL1**

CXCL1 expression has been demonstrated in platelets, but it is also expressed by endothelial cells, neutrophils, monocytes, and macrophages. Thus, platelets may not represent the major source of CXCL1. CXCL1 binds to CXCR2 and with lesser affinity to CXCR1. CXCL1 has been demonstrated to support arrest of human mononuclear cell lines and primary monocytes under flow conditions. This effect is mediated through CXCR2 signaling which was shown to be G Protein-mediated and may play an important role in monocyte recruitment to atherosclerotic lesions. Bovine aortic endothelial cells as well as HUVECs have been demonstrated to express CXCL1 when exposed to increased shear stress, which may promote monocyte arrest under flow. It remains unclear to what extent platelet-derived CXCL1 is involved in these events. Global absence of CXCL1 in Cxcl1<sup>−/−</sup> mice crossed with Ldr<sup>−/−</sup> atherosclerosis-prone mice resulted in smaller lesion size after 16 weeks of Western diet although serum lipids remained unchanged. Interestingly, chimeric Ldr<sup>−/−</sup> mice that received bone marrow from mice lacking CXCL1 did not show this reduction suggesting that chemokine expression within the aortic wall rather than in blood leukocytes is the determining factor. Similarly, a role for leukocyte CXCR2 expression was demonstrated, as Ldr<sup>−/−</sup> mice repopulated with bone marrow from Cxcr2<sup>−/−</sup> mice displayed smaller lesion size than those reconstituted with Cxcr2<sup>+/−</sup> bone marrow.

**Other Chemokines**

CCL2 is abundantly present in atherosclerotic lesions. In vivo, the importance of CCL2 has been shown by knocking out either its receptor CCR2<sup>−/−</sup> or CCL2 itself in mouse models of atherosclerosis (Apoe<sup>−/−</sup> or Ldr<sup>−/−</sup>), resulting in reduced atherosclerotic lesion size. In the vasculature CCL2 is mostly produced by endothelial cells and smooth muscle cells, but CCL2 presentation by platelets has been demonstrated to support monocyte adhesion in vitro and neointima formation in vivo.26

CCL3 (macrophage inflammatory protein-1α, MIP-1α) has been shown to be expressed in human atherosclerotic plaques. It binds to CCR1 and CCR5. CCL3 has been detected in the shoulder region of plaques, and its blood levels are elevated levels during acute myocardial infarction, suggesting a role for this chemokine in plaque destabilization. CXCL5 (encoding a neutrophil chemotactant peptide, ENA-78) has been cloned from human platelets. Having high structural similarity with other chemokines like NAP-2 or CTAP-III, CXCL5 was demonstrated to attract neutrophils. Different from CCL5 or CXCL4, preincubation of human microvascular endothelial cells with CXCL5 did not enhance monocyte arrest under flow. The relevance of this chemokine in atherogenesis remains unclear.

CXCL12 is present in atherosclerotic lesions. CXCL12 is expressed in platelets, but also in endothelial cells, smooth muscle cells, and macrophages. Apart from its potential function in platelet activation, CXCL12 seems to play an important role in neointima formation after arterial injury as it has been demonstrated to attract bone marrow–derived smooth muscle cell progenitors in Apoe<sup>−/−</sup> mice after wire injury. CXCL12 expression was increased on activated platelets, where it induced adherence of CD34+ progenitor cells in vitro under static conditions and under flow. CXCL12-mediated neointima formation was shown to depend on hypoxia-induced factor-1α (HIF-1α) as demonstrated by knock-down experiments in wire-injured Apoe<sup>−/−</sup> mice.

**Platelet Chemokines Induce Differentiation of Other Cells**

**CXCL4**

Several platelet chemokines including CXCL4 have been described to induce differentiation of other cell types. Apart from its potential role in conjunction with CCL5 in monocyte recruitment to the vascular wall, CXCL4 may promote monocyte differentiation to macrophages or specialized antigen-presenting cells (in the presence of IL-4 and/or GM-colony stimulating factor [CSF]). CXCL4-driven macrophages differ from their untreated counterparts by lack of HLA-DR and high CD86 surface expression. Furthermore, CXCL4 induces higher phagocytic capacity as compared to GM-CSF–induced macrophages. Accordingly, CXCL4 may increase atherogenesis by promoting differentiation of monocytes into macrophages and foam cells. In murine atherosclerotic plaque, CXCL4 is found close to foam cells. Macrophages and foam cells differentiated from monocytes under the influence of CXCL4 are phenotypically different from those differentiated in the presence of M-CSF. CXCL4 was demonstrated to inhibit binding and uptake of low density lipoprotein (LDL) through the LDL receptor, which may enhance oxidation of LDL and be related to a 10-fold increase in the amount of esterified oxidized (ox)LDL in macrophages, which would be expected to promote foam cell formation in atherosclerotic lesions.

Apart from its effects on monocytes, CXCL4 also exerts effects on other cell types including inhibition of cell proliferation and angiogenesis in endothelial cells. CXCL4 inhibits proliferation and cytokine release from activated T
cells in vitro.\textsuperscript{54} T cells exposed to CXCL4 show an inhibition of cell proliferation and deviation toward a T helper (Th2) phenotype.\textsuperscript{80} CXCL4 suppresses megakaryopoiesis in vitro and in vivo.\textsuperscript{81}

**CXCL7**

Apart from their chemotactic activity, some CXCL7 variants may influence differentiation and behavior of other cells. Thus, CXCL7 variants may promote synthesis of extracellular matrix components such as hyaluronic acid or glycosaminoglycans by fibroblasts.\textsuperscript{37} Interestingly, CXCL7 variants with a truncated C terminus (thrombocidins) have antimicrobial activity.\textsuperscript{82,83}

**CXCL12**

CXCL12 plays an important role in neointima formation after arterial injury.\textsuperscript{84} Apart from its role in recruiting progenitor cells to the site of vascular injury,\textsuperscript{44} it has also been demonstrated to promote differentiation of CD34+ progenitor cells to endothelial cells in vitro and in vivo.\textsuperscript{84} Thus, upregulation of the endothelial differentiation marker CD146 was inhibited in CD34+ progenitor cells if treated with antibodies against CXCL12 or its receptor CXCR4.\textsuperscript{85}

**Conclusions**

Platelets and platelet-derived chemokines play an important role in atherogenesis. In contrast to chemokines synthesized in response to certain stimuli, they are stored within the \( \alpha \)-granules of platelets and can be quickly released on platelet activation. Most chemokines are able to attract specific leukocyte subsets to the lesion site, but they also influence proliferation, differentiation, and degranulation of various cell types. Alone or as heterodimers, platelet-derived chemokines exert their effects via different G protein–coupled receptors expressed in their target cells, some of which remain to be identified. Some chemokines may regulate expression or processing of precursors of other chemokines suggesting a finely tuned chemokine network.

Recent studies have illustrated the clinical importance of gene polymorphisms\textsuperscript{86} or serum levels of circulating chemokines to predict clinically significant atherosclerosis\textsuperscript{85} or even acute cardiovascular events.\textsuperscript{22} These findings emphasize the importance of a good understanding of chemokine function in atherosclerosis. Some platelet-derived chemokines such as CXCL4 may represent interesting therapeutic targets as they promote several steps in the development of atherosclerotic lesions. Antiplatelet therapy has been a valuable tool to treat patients with atherosclerosis and has clearly been shown to prevent adverse events. Combining antithrombotic with anti-inflammatory treatments targeting platelet-derived chemokines may improve long-term prognosis in patients with cardiovascular disease.

**Disclosures**

None.

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