Platelet-derived chemokines in vascular biology

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Summary
Undoubtedly, platelets are key elements in the regulation of thrombosis and haemostasis. Along with their primary task to prevent blood loss from injured vessels, platelets have emerged as regulators of a variety of processes in the vasculature. Multiple challenges, from the contact and adhesion to subendothelial matrix after injury of the vessel wall, to interactions with blood cells in inflammatory conditions, result in platelet activation with concomitant shape change and release of numerous substances. Among these, chemokines have been found to modulate several processes in the vasculature, such as atherosclerosis and angiogenesis. In particular, the chemokines connective tissue activating protein III (CTAP-III) and its precursors, or truncation products (CXCL7), platelet factor 4, (PF4, CXCL4) and its variant PF4alt (CXCL4L1) or regulated upon activation and normal T cell expressed and secreted (RANTES, CCL5), have been investigated thoroughly. Defined common properties as their aptitude to bind glycosaminoglycans or their predisposition to associate and form homooligomers are prerequisites for their role in the vasculature and function in vivo. The current review summarizes the development of these single chemokines, and their cooperative effects that may in part be dependent on their physical interactions.

Keywords
Chemokines, platelet immunology, atherosclerosis, angiogenesis and inhibitors

Introduction
Platelets are anuclear cell fragments derived from megakaryocytes that contain a wealth of proinflammatory as well as (anti-)angiogenic mediators which play important roles in a variety of physiological and pathological conditions in the vasculature. The concept of platelets as immune cells has expanded the notion of platelets as major players in thrombosis and haemostasis, spurred by increasing discoveries on the repertoire of their inflammatory and immune-modulating molecules (1). Evidence for the particular importance of platelets in human atherosclerosis has been obtained from a conclusive prospective study that found an association between platelet concentration and aggregability and long-term incidence of fatal coronary heart disease in a population of apparently healthy middle-aged men (2). From the many platelet-derived substances, chemokines are an especially intriguing family of proteins that are stored in their α-granules and exert numerous biological activities. A variety of chemokines have been found to be expressed by platelets (Table 1) (1), however, CTAP-III/NAP-2 (connective tissue activating peptide III/ neutrophil activating peptide-2, CXCL7), PF4 (platelet factor 4, CXCL4), PF4alt (platelet factor 4 alternative, CXCL4L1) and RANTES (regulated upon activation and normal T cell expressed and secreted, CCL5) will be the focus of this review since they have been well studied and are released in considerable amounts. They share characteristic chemophysical properties, e.g. a positive charge and high affinity for negatively charged glycosaminoglycans which enables their retention on the surface of endothelial cells even in the presence of shear forces. Related to their capacity for immobilization on the endothelial surface distinct platelet chemokines seem to be adapted to conditions in the vasculature as their shear-resistant decoration of endothelial cell surfaces permits leukocyte arrest under flow conditions (3). Notably, platelet chemokines do not form only homo-aggregates, but also heterophilic interactions occur, which leads to significant modulation of their biological activ-
Chemokine Alternativen am eR eceptor System

ties (4, 5). These and other recently discovered interactions may represent regulatory mechanisms specific to a thrombocytic chemokine system.

**CXCL7**

Among the chemokines stored and secreted by platelets, CXCL7 is the most abundant representative. In fact, by appearing in the serum at micromolar concentrations (1.6 to 4.8 µM), CXCL7 surpasses most other platelet-associated chemokines up to several orders of magnitude, and even exceeds CXCL4, the second most frequent one (0.4 to 1.9 µM), by more than two-fold (16). In clinical trials and other studies where the amount of CXCL7 released into the blood is taken as a measure for platelet activation, CXCL7 virtually represents the only CXCL7 protein that may be addressed as a functionally typical chemokine, since it is the only variant capable of efficiently stimulating chemotactic migration and thereby recruitment to inflammatory sites of its target cells, namely neutrophil granulocytes (Fig. 1) (27). With regard to this, PBPA and CTAP-III may be addressed as inactive precursors of NAP-2, although they exhibit a variety of other biological functions independently of proteolytic processing.

**Processing and biological activities of CXCL7**

It has been reported that both CTAP-III and NAP-2, as well as other truncated CXCL7 variants of intermediate size, all have the capacity to support various aspects of fibroblast metabolism, e.g. synthesis of matrix components, such as hyaluronic acid and glycosaminoglycans (GAGs) (28), enhancement of GLUT-1 glucose transporter expression and concomitant glucose uptake (29). While these and other activities support the notion that CXCL7 as a platelet-derived mediator may participate in reparative functions subsequent to vascular tissue injury, its potential role as a growth factor has remained controversial. Thus, some years ago fibroblast mitogenic activity was reported for a leukocyte-secreted protein corresponding to full-length pro-PBP, this kind of activity being absent from the shorter CXCL7 variants (30). Others were unable to reproduce these findings with respect to both the secretion of pro-PBP by monocytes and the mitogenic activity of recombinant pro-PBP, although proteolytically processed CXCL7 derivatives were being formed (31). In fact, many of CXCL7’s cell-directed regulatory activities have been observed to be preferentially mediated by the smaller

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<th>Chemokine</th>
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**CXCL7 variants emerge by proteolytic cleavage**

The abundance of CXCL7 variants appears to follow a certain sequence of proteolytic events during platelet generation and activation. Thus, the major variant in megakaryocytes is PBP, along with a minor proportion of CTAP-III. As maturation proceeds and platelets become formed, the proportion of the shorter variant CTAP-III increases while that of PBP drops to about 25% (21). Following platelet activation most of the CXCL7 released into the blood is made up of CTAP-III, with PBP accounting for only about 10% (21). The proteases that are responsible for this conversion are still unknown. It has been reported that further proteolytic processing of these released precursors generates β-TG (22). However, according to our experience β-TG is virtually absent from release supernatants of purified thrombin-stimulated platelets, as is NAP-2, the shortest CXCL7 truncation product. In fact, we and others could show that NAP-2 is only formed in the presence of leukocytes or chymotryptic leukocyte-derived proteases, which have the capacity to cleave platelet-released PBP and CTAP-III behind the single tyrosine residue present in CXCL7 (23–26). NAP-2 virtually represents the only CXCL7 protein that may be addressed as a functionally typical chemokine, since it is the only variant capable of efficiently stimulating chemotactic migration and thereby recruitment to inflammatory sites of its target cells, namely neutrophil granulocytes (Fig. 1) (27). With regard to this, PBP and CTAP-III may be addressed as inactive precursors of NAP-2, although they exhibit a variety of other biological functions independently of proteolytical processing.

Table 1: Chemokines known to be stored in platelets.
CXCL7 variants, especially by NAP-2. Interestingly this chemokine, which represents a highly proinflammatory end product of limited CXCL7 proteolysis after platelet activation, may act back on megakaryocyte development as part of a negative feedback loop (32, 33), in that it suppresses polyploidization and proplatelet formation during megakaryocyte maturation (34). These inhibitory effects are obviously specific, since no decreases in colony formation of erythroidicastic or granulocyte-macrophage progenitors were found (35). Recently, monocyte-derived NAP-2 produced in a narrow microenvironment has come into play as an additional source for megakaryocyte regulation (31).

**CXCR1 and CXCR2 are receptors for NAP-2**

The expression of the two chemokine receptors CXCR1 and CXCR2 on megakaryocytes (36) suggests that NAP-2 affects maturation of these cells via signalling through its cognate G protein-coupled receptors. In fact, we and others have shown that efficient interaction of CXCL7 proteins with these receptors and concomitant cell activation, as seen with human neutrophils, requires their N-terminal truncation down to a molecular size not essentially larger than that of NAP-2 (23, 24, 26). On the other hand, further truncation entails dramatic loss in biological activities because it destroys an aa-sequence motif (ELR) common to all neutrophil-stimulating CXC-chemokines and of crucial importance for their interaction with CXCR1 and CXCR2 (37, 38). However, while an extended N-terminus in most ELR-CXC chemokines has only moderate functional impact, the N-terminus in CXCL7 proteins like CTAP-III has been shown to fold back over the ELR motif, thereby preventing proper interaction of the chemokine with its receptors (39).

**Regulation of CXCL7 activity**

The strict requirement for removal of the inhibitory N-terminus for CXCL7 to become active as neutrophil-directed chemokine constitutes the basis for its potential role as an intra- and extravascular regulator of neutrophil recruitment and activation. As we have shown, CXCL7 processing to NAP-2 occurs within minutes and is mainly catalyzed by neutrophils, the NAP-2 target cells themselves, without significant contribution of other blood leukocytes (26). Processing by neutrophils does not require cell activation, but occurs through serine protease cathepsin G bound to the extracellular membrane. Interestingly, plasma inhibitors of serine proteases are not effective to control processing in mixed thrombi of leukocytes and platelets (own unpublished data). Instead, we have evidence for the involvement of two other control mechanisms, one of which relies on CXCL4, another major platelet chemokine, which we found to inhibit CXCL7 processing by cathepsin G (5). A more sophisticated mechanism relies on the fact that NAP-2, while being formed during processing, may interact with and downregulate its high-affinity receptor CXCR2 from the neutrophil surface at substimulatory concentrations and thereby homologously desensitize processing cells to NAP-2 as well as other ELR-CXC chemokines (26, 40). Interestingly, we found processing of platelet-secreted PBP to occur almost one log faster than that of CTAP-III, rendering the former protein an especially efficient inhibitor of neutrophil activation, affecting chemotaxis as well as degranulation (41). As seen with CTAP-III in another study, desensitization of CXCR-2 affected neutrophil adhesion to endothelium as well as transendothelial migration (42). Moreover, CXCL7 appears to form part of a regulatory system preventing premature and potentially harmful neutrophil degranulation in response to other early vascular mediators of inflammation: it exhibits heterologous desensitization of neutrophil degranulation induced by endothelial cell-derived mediators such as PAF (platelet activating factor) and IL-8 (CXCL8), which are formed upon wounding, but does not affect C5a, a component only formed upon direct contact with invading microorganisms (26, 41, 43). While desensitization evidently is mediated through CXCR2, the NAP-2 low-affinity receptor CXCR1, binding the chemokine about 100-fold less efficiently (41, 44, 45), appears to be required for efficient neutrophil recruitment along extended NAP-2 gradients (nanomolar to micromolar) likely to occur in occluded blood vessels. Although most chemokines induce chemotactic cell migration with a single biphasic kinetic encompassing a relatively narrow range of concentrations, NAP-2 may address its two receptors in sequence and continue cell attraction through CXCR1 at high concentrations where CXCR2 has already undergone desensitization (46). As seen recently, NAP-2 might also be responsible for neutrophil accumulation around vascular tissues as a consequence of mast cell activation. Mast cells which are found in the tissue closely associated with blood vessels secrete various proteases upon stimulation, among which we found chymase to very efficiently process CTAP-III to NAP-2. Mast cells from human

![Figure 1: Biologic activity of the platelet-derived chemokines CXCL7, CXCL4 and CCL5. Depicted here is a scheme that visualizes the main activities as well as cooperation and mutual regulatory interaction among the major platelet-secreted chemokines.](image-url)
skin and lung were similarly active, and as with neutrophils, processing by these cells was under the inhibitory control of CXCL4 (5).

CXCL7 proteins may also occur as variants bearing limited truncations by 2 to 7 aa at the C-terminal. We found some of these variants (deleted by 4 to 7 residues) to arise in mixed cultures of platelets and leukocytes (47, 48), while others (deleted by 2 residues) were extractable from platelet granules (49). In general, these variants exhibited enhanced stimulating activity as well as desensitizing activity for neutrophil functions, which suggests a regulatory role under certain inflammatory conditions (47, 48). However, as a most unexpected finding we discovered that these C-terminal variants also exhibited direct antimicrobial activity against gram positive and negative bacteria, as well as against some fungi (49). In a model of infectious endocarditis it could be shown that these platelet-derived “thrombocidins” indeed played an essential role as a defense factor in this kind of disease (50). On the other hand, the increased levels of NAP-2 associated with acute coronary syndromes are suspected to induce inflammatory responses within the atherosclerotic plaque, which could lead to plaque rupture (140). Thus, as with most chemokines, the protective and adverse functions of the different CXCL7 proteins will still require thorough investigation.

**CXCL4, CXCL4L1**

Platelet factor 4 (PF4/CXCL4), which was discovered in 1977 (52), was the first member of a large group of cytokines that were later subsumed in the family of chemokines. PF4 was originally described to induce a chemotactic response in neutrophils and monocytes at rather high concentrations (53, 54). However, these findings could not be confirmed by later studies (27, 55), and today it is clear that highly purified PF4 lacks chemotactic activity for neutrophils, monocytes, T cells, or any cell population tested so far (56–58). The controversial results reported in the past may be explained by small contaminations of other chemokines, identified later on as RANTES and NAP-2 (14, 59, 60), in PF4 preparations at that time.

Although PF4 has not only been found in megakaryocytes and platelets but also in different cell types, such as smooth muscle cells or macrophages (61, 62), relevant amounts only occur in platelet α-granules which become massively released within minutes of platelet activation. As has been reported for CXCL7, PF4 plasma levels are below 1 nM, while normal serum contents reach more than a thousand-fold higher concentrations (1–2.5 μM; (16, 63, 64). Most recently, the product of a non-allelic gene variant termed PF4alt (CXCL4L1) (65, 66) with only three aminoacid substitutions in the C-terminal α-helix has been isolated from platelets as well (8). Just recently, the amino acid divergence in the signal peptide region has lead to experiments that convincingly show a distinct subcellular localization. In contrast to PF4, which is stored and released upon activation, PF4alt appears to be continuously synthetized and secreted through a constitutive pathway (62).

**Receptors and intracellular signal transduction**

Even in terms of its receptors, PF4 is in many ways an unusual chemokine. One of the first properties which could be assigned to PF4 was its capacity to bind to charged GAGs, the carboxy-

brate side chains of proteoglycans. Although proteoglycans are mostly understood as bystander molecules or coreceptors, an increasing body of evidence has accumulated suggesting that proteoglycans, most likely in association with a protein signalling chain, may serve as a functional receptor for PF4. We have shown in the past that PF4 specifically binds to an integral chondroitin sulfate proteoglycan of 220 kDa on the surface of PMN, and that the addition of free GAG chains as well as the removal of cell-associated chains significantly block PF4-binding to neutrophils as well as PF4-induced signal transduction and cell activation (67, 68). PF4-signaling is not accompanied by intracellular calcium transients and is insensitive to Gi protein inhibitors like pertussis toxin (56–58). In a most recent study, we could identify three members of the family of src-kinases as well as monomeric GTPases and the MAP kinase JNK as essential signalling elements activated by PF4 in PMN (68, 69). In an outstanding study in 2003, Lasagni et al. identified an alternative splice variant of the CXCR3 (termed CXCR3B) as a second receptor for PF4 (70). While Mig (CXCL9), IP10 (CXCL10), and I-TAC (CXCL11) are able to bind to both splice forms of CXCR3, PF4 selectively interacts with CXCR3B. Intriguingly, CXCR3B, which is expressed on endothelial and T cells, but not on monocytes or neutrophils, is not coupled to Gi but to Gs proteins, leading to the formation of cAMP and activation of PKA (58, 70). Most likely, this receptor plays a central role in PF4-mediated inhibition of endothelial cell proliferation and chemotaxis. However, binding to at least two biochemically and functionally different receptors may constitute the molecular basis for the heterogeneity of PF4 functions on various cell types.

**Direct effects on cellular functions**

The rapid mobilization of PF4 together with RANTES and the NAP-2-precursor CTAP-III after platelet activation place these chemokines in a role as first-line mediators in the control of an early host defence. This inflammatory response is initially dominated by an innate immune response, especially by the activation of neutrophils and monocytes. Since CTAP-III, after having been proteolitically processed by neutrophil proteases to NAP-2 (Fig. 1), serves as a potent activator of neutrophils and RANTES acts as a strong chemotaxin for monocytes, the question remains – which functions are finally left for PF4?

The answer to this question is that nearly all cells in the vasculature are affected by PF4 (Fig. 1); however, the corresponding cellular functions which are induced or regulated in these cells are not typical for chemokines. Interestingly, depending on the cell type targeted, PF4 has the capacity to promote activating as well as deactivating cellular functions involved in acute host defense as well as long-term regulatory processes (Table 2). Han et al. reported that PF4 supports the survival of hematopoietic stem cells as well as of progenitor cells (71) and suppresses the development and maturation of cells from the megakaryopoietic lineage (32). Furthermore, an anti-proliferative activity of the chemokine on endothelial cells and fibroblasts was reported by several authors (72–75). Inhibition of angiogenesis, which may explain the tumorostatic property of the chemokine (76), appears to be rather complex in regulation and also involves the direct regulation of cellular functions by a PF4 receptor-mediated process.
PF4 was shown to play an essential role as a first and second line mediator in the host defence against microbial invaders. Immediately after contact with PF4, monocytes and macrophages display a strong and long-lasting generation of reactive oxygen metabolites as well as an increased capacity for unspecific phagocytosis (58). However, in a second phase of activation, PF4, like GM-CSF or M-CSF, prevents monocytes from undergoing apoptosis and induces monocyte differentiation (Fig. 1) into macrophages (79). Phenotype and functions of these PF4-derived macrophages appeared to be rather unusual and differ remarkably from those induced by the other two cytokines. Most intriguingly, macrophages generated by PF4 are characterized by a lack of surface-expressed HLA-DR antigen and by their inability to activate T lymphocytes (unpublished observations). The development of a high capacity for the uptake and killing of microorganisms (58) accompanied by a substantial release of TNF and proinflammatory chemokines like IL-8, MIP-1α, or MIP-1β ([79] and unpublished results), indicates that these cells are highly specialized for inducing and maintaining an innate immune response. These observations are supported by recent findings which describe a direct inhibitory effect of PF4 on the activation of T cells. By using different antigen-specific as well as polyclonal T cell-stimulation models we and others could demonstrate that the chemokine acts as a potent suppressor of T cell function in terms of reducing lymphoproliferation as well as the release of IL-2 and IFN-γ (57, 80).

Cooperative biological activities

One of the most intriguing properties of PF4 is its capacity to cooperate with other stimuli resulting in synergism of cellular functions, modulation of biological processes, or the generation of novel cell types. One example of such a cooperative principle is the generation of NAP-2 by neutrophils and mast cells (Fig. 1). As previously mentioned, the generation of NAP-2 from the corresponding precursor molecules PBP and CTAP-III by neutrophil cathepsin G or mast cell-derived chymase was found to be under the inhibitory control of PF4 (5). On the other hand, PF4 significantly enhances neutrophil adhesion to endothelial cells induced by NAP-2 (42). Furthermore, on monocytes, RANTES provokes a strong chemotactic response; however, it is only a rather weak inducer of functions directly involved in the uptake and killing of microorganisms (81). In contrast, PF4 lacks chemotactic activity but was shown to be an effective inducer of phagocytosis and oxygen radical formation (58). Simultaneous exposure of monocytes to PF4 and RANTES, which may reflect the physiological situation when these cells become encountered by degranulating platelets, results in a strong synergistic enhancement of the latter functions. Furthermore, PF4 drastically enhances RANTES-induced monocyte arrest on endothelial cells under flow conditions (82) which is based on a heterophilic interaction between both chemokines.

Cooperation of PF4 with regulatory cytokines on monocytes initiates differentiation into physiologically different cell populations, which enables a high degree of flexibility of the host response in an inflammatory situation. While treatment of monocytes with PF4 alone leads to the development of activated macrophages, it has been shown most recently that a combination of PF4 and IL-4 induces differentiation into professional antigen-presenting cells (APCs). PF4-derived APCs were clearly distinct from conventional dendritic cells and macrophages. Functional analyses revealed that these APCs induced substantial proliferation of lymphocytes and cytotoxicity of natural killer cells while they provoked only modest cytokine responses when compared with conventional (GM-CSF/IL-4-derived) DCs (83). Thus, by generation of either macrophage or APC cell types, PF4 may...
play a pivotal role in regulating the balance between innate and adaptive immune response already at a very early stage of inflammation.

CCL5

In contrast to PF4 and NAP-2, RANTES is a member of the CC-branch of the chemokine family. In search of T cell-specific genes, the coding sequence of RANTES has been found in a subtracted cDNA library isolated from a functional, non-transformed, antigen-stimulated T-cell line (89). It became apparent that RANTES is not uniquely expressed by T cells through the discovery of a protein with chemotactic activity present in the releasate of thrombin-stimulated platelets which could be identified as RANTES by NH$_2$-terminal amino acid sequence analysis and mass spectrometry (14). Immunocytochemistry detected RANTES and for the first time also MIP-1 alpha within the alpha-granules of human platelets (13). Since then it became clear that many more cell types, such as fibroblasts, epithelial cells, and mesangial cells, express this proinflammatory molecule upon activation with certain stimuli like IFN-γ and TNF-α. In contrast to PF4, RANTES exerts a less pleiotropic but marked biologic activity accentuated on recruitment of monocytes, T cells and eosinophilic granulocytes (14, 90, 91). At the level of tertiary structure chemokines share a similar monomeric fold, characterized by a disordered amino-terminal domain, followed by a conserved core region, consisting of three anti-parallel β-strands and a carboxyl-terminal α-helix. The quaternary structure of RANTES as a typical CC-chemokine is very different from that of the C-X-C chemokines since dimer formation occurs mainly at the mobile N-terminal regions, whereas CXC-dimers are assembled by the extension of their antiparallel beta-sheet domains (92). In vitro forms RANTES at physiological pH higher order aggregates, however, in vivo the quaternary structure remains unknown (93).

Receptors and intracellular signal transduction

A receptor with a high affinity for both MIP-1α and RANTES has been deduced by steady-state radioligand competition and desensitization assays and was shown to be G-protein linked and to induce transient calcium influx (94, 95). The first CC chemokine receptor cloned turned out to be the receptor for RANTES/MIP1-α (96, 97), and was designated CCR1 according to the receptor nomenclature system (98). RANTES-receptors, which cause selective chemotaxis of eosinophils and basophils, were designated CCR3 and CCR4, respectively (99, 100). The last cloned chemokine receptor for RANTES was designated CCR5 and detected constitutively in monocytes but not in primary neutrophils or eosinophils and is also a seven transmembrane G-protein-coupled receptor (GPCR) (101, 102). The identification of RANTES, MIP-1 alpha, and MIP-1 beta as HIV-suppressive factors (103) lead to the discovery that CCR5 is a crucial host factor exploited by HIV for cell entry (104–106).

Investigation of the signal transduction mechanisms induced by RANTES in T cells revealed a potentially important role for RANTES in the generation of T-cell focal adhesion and subsequent cell activation via a molecular complex containing FAK (focal adhesion kinase), the tyrosine kinase zeta-associated protein (ZAP) 70 and paxillin (107).

Truncation of the N-terminal part of RANTES results in the development of antagonists with decreased receptor specificity indicating that NH$_2$-terminal residues partly determine the receptor specificity of RANTES. Deletions within this region permit binding to multiple chemokine receptors (108). Cleavage of the first two N-terminal amino acids by dipetidyl peptidase IV (CD26) results in a variant that lacks activity for CCR1 but retains that for CCR5 (109). Apart from their distinct expression on leukocyte subtypes RANTES-receptors CCR1 and CCR5 mediate differential functions, as RANTES-induced arrest of lymphocytes and monocytes is mediated predominantly by CCR1, whereas CCR5 mainly contributes to the spreading in shear flow (110). However, the exact mechanisms how RANTES interacts with its receptors leading to chemotaxis and cell arrest are presently unknown.

Biologic activity

At nanomolar concentrations RANTES behaves as a typical chemokine causing chemotaxis of mononuclear cells by inducing cell polarization and transendothelial cell migration (TEM). Transendothelial migration of monocytes and lymphocytes is integrin-dependent and requires adhesion molecules from molecules of the immunoglobulin superfamily, such as ICAM-1 and VCAM-1 (111, 112). Leukocyte migration from the blood into tissue requires activation signals since integrins in the resting conformation only have low affinity to their ligands. Chemokines have been shown to be good candidates for integrin activation as they are released during inflammation and trigger adhesion in all kinds of leukocyte subsets. It has been suggested that RANTES plays a specific role in the adhesion of monocytes and lymphocytes as intracellular integrin-domains are phosphorylated shortly after exposure to RANTES (113). So far, the exact mechanism that causes RANTES-induced integrin dependent adhesion and TEM has not been fully elucidated.

Besides inducing cell migration, RANTES acts at high concentrations independently of its G-protein-coupled receptors as T-cell mitogen and induces the release of proinflammatory mediators (107, 114, 115). Its arrest functions are linked with its peculiar capacity to form large homotypic aggregates and its high affinity for GAGs present on the surface of endothelial cells, basement membrane and extracellular matrix that allows RANTES to be immobilized and to induce shear resistance arrest (3, 116, 117).

General predictions for the chemokine interface for interaction with GAGs can not be made. The heparin-binding region of PF4 is associated with residues located in the C-terminal helix, as well as in the loops of the beta-sheets, in such a way that a PF4 tetramer will display a belt of positively charged residues around the entire molecule (118). In the RANTES molecule two clusters of basic amino acids present conceivable heparin-binding motifs in the 40s loop between the second and third β-strand and at the C-terminal α-helix. Extensive studies with single amino acid-mutated variants could clearly show that only the basic residues in the 40s loop are responsible for the specific and high affinity of RANTES to different types of GAGs (119, 120).
In vitro, RANTES mutants that either lack the ability to form oligomers or are deficient in GAG-binding retain their chemotactic activity (119, 120). However, they are unable to recruit cells when administered intraperitoneally (121, 122). Notably, different from chemotaxis, efficient leukocyte arrest on endothelium seems to be dependent on the formation of RANTES oligomers to bridge surface-bound RANTES and CCR1 (123). The selective binding of chemokines to subsets of glycosaminoglycans on the cell surface induces polymerization facilitating their binding to receptors and enhancing their effects on high-affinity receptors within the local microenvironment and may establish an immobilized gradient leading to haptotaxis (124–126). In addition, structural motifs that are required for the oligomerization of RANTES are important for the heterophilic interaction of RANTES with PF4 which increases surface immobilization and enhances monocyte adhesion to endothelial cells under flow conditions (Fig. 1) (82).

Implications of platelet-derived chemokines in vascular biology

Data from animal models provide evidence for the crucial role of activated platelets in the pathogenesis of atherosclerosis beyond acute atherothrombotic events (127, 128). A variety of adhesion molecules enable activated platelets and platelet-derived microparticles to interact transiently with inflammatory altered endothelial cells and atherosclerotic lesions or lesions induced by wire injury (12, 117, 129). Monocyte recruitment into the subendothelial arterial space is an early step in the pathogenesis of atherosclerosis. Dependent on their affinity to GAGs, platelet chemokines such as RANTES get deposited on the luminal lining where they activate monocytes which has been shown in vitro and ex vivo (123, 130). Met-RANTES, an antagonistic RANTES-derivate has been applied in an atherosclerosis-prone transgenic mouse model and reduces the development of atherosclerotic lesions (131). In humans, the role of RANTES is less defined. We know that a promotor polymorphism for the RANTES gene results in increased transcriptional activity and is associated with coronary artery disease (CAD) independently of conventional risk factors (132). In contrast, in a case-control study serum levels of RANTES appeared to be lower compared to healthy controls (133). Up to now, the role of PF4 and NAP-2 in atherosclerosis have not been evaluated intensely. Elevated plasma concentrations of PF4 and β-TG occur in patients with CAD or peripheral vascular disease, and platelets of patients with atherosclerosis exhibit a reduced intraplatelet content of PF4 which may reflect enhanced in-vivo platelet activity and give a first hint of their relevance in atherosclerosis (134–136). PF4 and, to a lesser extent, NAP-2 have been found to be associated with human atherosclerotic plaques (137). Furthermore, PF4 takes part in the metabolism of atherogenic lipids, such as low-density lipoprotein (LDL)-derived cholesterol and oxidized LDL (oxLDL) that accumulate during the course of atherogenesis in macrophages of the arterial wall. The addition of PF4 to cultured cells resulted in increased retention of LDL on cell surfaces by inhibition of LDL-receptor-dependent binding, internalization, and degradation of LDL and binds oxLDL directly (138, 139).

Patients with stable, and particularly those with unstable, angina exhibit markedly raised plasma levels of NAP-2 compared with control subjects, which can be reduced by platelet aggregation inhibitors such as aspirin (140). PF4 may play an important role as well in the acute coronary syndrome where plaque rupture leads to atherothrombosis. Over a narrow range of concentration PF4 itself promotes thrombosis. However, the exact model (cell surface charge neutralization, binding to (anti)thrombotic substances), of how PF4 affects thrombosis has not been clarified ultimately and is discussed in detail by Lambert et al. in this Theme Issue (141). Another evolving role for platelet chemokines in the vasculature relates to the process of angiogenesis that may be beneficial in wound healing but also detrimental in pathological conditions such as cancer and atherosclerosis. Capillary sprouting and endothelial cell proliferation can be induced by platelet releasates in vitro and by isolated platelets, which has been ascribed to potent angiogenesis inducing factors, e.g.VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor), and PDGF (platelet-derived growth factor) (142, 143). Interestingly, NAP-2 induces CXCR2-dependent firm arrest of endothelial progenitor cells (EPCs) on fibronectin, fibrinogen, and on platelet-coated endothelial matrix accelerating endothelial cell recovery after wire injury and possibly developing angiogenic activity (144). On the other hand, the platelet products PF4 and PF4alt are potent inhibitors of angiogenesis. Several mechanisms may account for this: PF4 prevents binding of VEGF to its receptor KDR/flk-1 (74), and inhibits the mitogenic activity of bFGF (73). Last but least PF4 can exert its angiostatic activity through CXCR3B (70). How platelets regulate the activity of their oppositely acting secretory products will have to be determined.

These studies substantiate the outstanding role that platelet-derived chemokines play in vascular biology. However, neither of them is solely expressed by platelets such that platelet-specific animal models are needed to further dissect their role in vivo.

Concluding remarks

In conclusion, platelets can be designated as a well equipped first aid box, where we find everything necessary to survive an acute case of emergency. This includes, apart from factors that activate the clotting system to stop blood flow and to immediately isolate the site of infection, a set of chemokines which act as precisely coordinated tools in the combat against microbial invaders. By acting in a cooperative manner, the three chemokines RANTES, CTAP-III/NAP-2, and PF4 are sufficient to induce a broad spectrum of host defence mechanisms reaching from an initial recruitment and activation of phagocytes to the control of long-lasting immune responses.


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