Platelet chemokines in health and disease

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Summary
In recent years, it has become clear that platelets and platelet-derived chemokines, beyond their role in thrombosis and haemostasis, are important mediators affecting a broad spectrum of (patho)physiological conditions. These biologically active proteins are released from \( \alpha \)-granules upon platelet activation, most probably even during physiological conditions. In this review, we give a concise overview and an update on the current understanding of platelet-derived chemokines in a context of health and disease.

Keywords
Platelet immunology, chemokines, atherosclerosis, inflammation, infectious diseases

Introduction
Platelets are small, discoid shaped anuclear cell fragments, 2-3 \( \mu \)m in diameter, which are derived from proplatelets, extending and being released into the bone marrow sinusoids (1). In recent years, a growing body of evidence has emerged considering platelets to be responsible not only for haemostasis and thrombosis but also to be important for immune- and inflammatory (patho)physiological conditions (2-4). Upon platelet activation, platelets release numerous chemokines from their \( \alpha \)-granules that regulate a wide spectrum of biological processes (5, 6) (Table 1 and Figure 1). Recent studies have suggested the existence of morphologically different and highly compartmentalised \( \alpha \)-granules subclasses (7) with spatial segregation and variable cargo, which has been proposed to be differentially released (8-10).

Chemokines are chemotactic cytokines, classified into four families, CXCL, CC, CX3C and XC, according to the arrangements of the cysteine residues participating in the formation of disulphide bridges, activating seven transmembrane G-protein coupled receptors that are classified according to their ligands (11-13). Furthermore, chemokine receptors may bind several different chemokines and the other way around (Table 1).

While the most abundant platelet-derived chemokines are still represented by CXCL4 (platelet factor 4/PF4) and CXCL7, a new non-allelic variant of CXCL4 termed CXCL4L1 (PF4alt) has been identified to be released by platelets and thereafter to be abundantly expressed on protein and mRNA level (5, 14) (Table 1). Whereas CXCL4 and CXCL7 have been identified as markers for the megakaryocytic lineage, other platelet chemokines or chemokine like mediators such as CCL5 (Regulated on Activation, Normal T cell Expressed and Secreted/RANTES), Macrophage Migration Inhibitory Factor (MIF) (94, 95), CXCL12 (stromal cell-derived factor 1/SDF-1) and CXCL5 (epithelial neutrophil-activating peptide/ENA-78) are abundantly expressed and released by platelets but have been primarily identified in other cell types.

Chemokines, which have been detected in platelets are ordered in Table 1 according to their protein expression level measured by quantitative proteomics (15), transcriptomics (16) and immunologic assays. For some of these chemokines, especially for the weakly expressed, there are no confirmative data about their actual release and biological role related to platelet activation.

Hence, in this review we will focus on the platelet-derived chemokines that have been most extensively investigated, thereby addressing their potential relevance in the context of health and disease.
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Table 1: Expression profile of chemokines in human platelets. The expression of chemokines in resting human platelets has been detected and quantified using different techniques. Due to the improved quality of proteomic approaches over the last years, we extracted the identified chemokines and their copy numbers per platelet from the most recent and detailed approach from Burkhard et al. (15). This has been complemented by the chemokine expression levels of transcripts, identified by sequencing the platelet transcriptome by Rowley et al. (16). In order to compare the respective chemokine abundance, we added the expression levels of actin, P-selectin and GPIIb/IIIa, as examples of important and highly expressed platelet proteins. Additional chemokines, detected by ELISA-arrays and expressed as number of molecules per resting platelet, have been included (93). ND (not detectable), NA (not available), RPKM (reads per kilobase of exon model per million mapped reads).

<table>
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<tr>
<th>Protein</th>
<th>Gene</th>
<th>Proteome: copy numbers per platelet</th>
<th>Transriptome: RPKM</th>
<th>ELISA Array: molecules per platelet</th>
<th>Cognate chemokine receptors</th>
<th>Platelet-dependent pathology</th>
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<tr>
<td>CXCL4 (Platelet factor 4)</td>
<td>PF4</td>
<td>563.000</td>
<td>7.223</td>
<td>132.756</td>
<td>CXCR3</td>
<td>angiostasis, cancer, megakaryocytopoiesis, HIT, ALI, malaria, HIV, atherosclerosis</td>
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<td>CXCL7 (Platelet basic protein)</td>
<td>PPBP</td>
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<td>CXCR2, DARC</td>
<td>neoplasia, megakaryocytopoiesis, antimicrobial activity</td>
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<td>CXCL4L1 (PF4alt)</td>
<td>PF4V1</td>
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<td>Macrophage Migration Inhibitory Factor (MIF)</td>
<td>MIF</td>
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<td>7</td>
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<tr>
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<td>23</td>
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<td>CXCR2, DARC</td>
<td>ALI, HIV, atherosclerosis, myocardial infarction</td>
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<tr>
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<td>5.667</td>
<td>21.754</td>
<td>CCR1, CCR5, CCR3, CCR4</td>
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<td>3.202</td>
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<td>CXCL16</td>
<td>CXCL16</td>
<td>ND</td>
<td>9</td>
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<td>CXCL8 (IL-8)</td>
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<td>ND</td>
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<td>CXCR1, CXCR2, DARC</td>
<td>HPC proliferation and differentiation</td>
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<td>ND</td>
<td>3</td>
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<td>Actin, cytoplasmic 1</td>
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<td>SLP</td>
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Vascular repair and angiogenesis

In addition to mediating inflammatory processes by activation and recruitment of leukocytes from the circulation, platelet chemokines were also recognised to participate in vascular repair in angiogenesis, physiological processes constantly occurring during renewal and regeneration. In this context, Massberg et al. described in a very elegant study that activated platelets secrete chemokine CXCL12 and provide the critical signal that recruits bone marrow (BM)-derived progenitors of smooth muscle cells (SPCs) and endothelial cells (EPCs) to sites of arterial thrombi (17), thereby initiating vascular remodelling. Platelet-derived CXCL12 was shown to induce in addition EPC adhesion on platelets via CXCR4 and EPC differentiation to endothelial cells (ECs), further promoting regeneration (18). By modulating plasma CXCL12 levels, the CXCR4 antagonist AMD3100 acutely promoted, while chronic AMD3100 treatment inhibited mobilisation of proangiogenic cells (19). Furthermore, platelets contain a number of mediators with opposite effects on angiogenesis. It has been controversially discussed about the storage of these mediators in heterogeneous subsets of granules and their different release (9, 20, 21). One of the most prominent examples is the chemokine CXCL4, which has been identified as a major angiostatic component due to its inhibition of growth factor-stimulated EC proliferation (22). Surprisingly, thrombin-activated platelets and their releasate have been demonstrated to exert a trend to a net proangiogenic effect due to the angiogenic factors vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and heparanase despite the presence of CXCL4 (23). CXCL4 blocked VEGF-induced angiogenesis by a direct interaction with VEGF which prevented receptor binding and signal transduction as well as a decrease in cellular VEGF production (24). Furthermore, mutants of CXCL4 which have a low affinity to heparin-like glycosaminoglycans (GAGs) have an unaltered angiostatic activity in analogy to the non-allelic variant CXCL4L1, which differs in only three amino acids within the C-terminus, resulting in a low affinity to heparin due to an alternative orientation of the C-helix but potent angiostatic activity (14, 25-27). The finding that both CXCL4 and its variant can bind and activate CXCR3 receptor is another mechanism mediating the induced angiostatic signals in ECs (28, 29).

Taken together, platelet chemokines can have opposed roles in vascular repair and angiogenesis, although the responsible mechanisms are still not fully understood.

Neoplasia

The role of chemokines in neoplastic disease has been extensively investigated in the last decades. The chemokine system is not only an important regulator of cell migration into and out of the tumour microenvironment but also in survival, proliferation, differentiation and angiogenesis, playing a major role in cancer biology (30). Irrespective of the kind of tumour, patients with metastatic disease showed increased platelet counts and a significantly elevated percentage of activated platelets in line with many previous observations (31). Various mechanisms underlying platelet activation during tumourigenesis and metastasis have been considered. The hypoxic state in the tumour environment induces tissue-factor production and later thrombin generation, thereby activating platelets. Furthermore, platelets may be activated within pathological vessels of the tumour due to contact with subendothelial matrix structures and the non-laminar blood flow (32). Since platelet chemokines are released after platelet activation, it is plausible that the progression of tumours may be influenced.

Of interest, CXCL4 has been proposed as a biomarker in cancer since it has been found to be elevated in platelets and serum samples both in early tumour growth in xenotransplanted tumours in mice and in human pancreatic and colorectal cancer (8, 33, 34). In contrary, CXCL4 and CXCL7 were significantly decreased in serum samples from patients with advanced myelodysplastic syndrome, often progressing to acute myeloid leukaemia (35). In line with its angiostatic and antiproliferative effects, CXCL4 caused a significant reduction in microvessel densities in myeloma xenografts and markedly reduced the tumour volume in vivo (36). Furthermore, CXCL4L1 more efficiently reduced tumour growth and the number of small blood vessels in animal models of melanoma and lung carcinoma than CXCL4, and efficiently prevented metastasis to various organs (37).

Apart from the evidence implying CXCL4 and its variant as endogenous anti-tumour mediators, data about the influence of the other platelet chemokines on neoplasia are scarce.

Regulation of megakaryo- and thrombopoiesis

First data, describing the role of platelet chemokines in haematopoiesis, were from 1990. Platelet CXCL4 and CXCL7 which is processed in platelets from platelet basic protein (PBP) through connective tissue-activating peptide-III (CTAP-III) further to β-thromboglobulin (BTG) have been shown to downregulate normal and pathologic megakaryocytopoiesis in vitro (38). One of the possible mechanisms, shown to be effective also in vivo, was the binding to low-density lipoprotein receptor–related protein 1 (LRP1) on megakaryocytes (39, 40). Additionally, CXCL4 promoted the adhesion of haematopoietic progenitors (HPCs) to stromal cells and consecutively inhibited the cell-cycle. In the same study it was shown that CXCL4 inhibited CXCL8 signalling most probably due to heterodimer formation (41). Similar to CXCL4, platelet-released CXCL8 inhibited the proliferation and differentiation of CXCR1- and CXCR2- positive HPCs to megakaryocytes (42).

In addition, the opposite role of CXCL12 as an inducer of megakaryo- and thrombopoiesis has been described. CXCL12 operated alone and in combination with thrombopoietin, increasing megakaryocyte colony formation (43) and acted as a potent chemotactic factor for mature megakaryocytes, mediating their migration through bone marrow ECs via CXCR4 (44). Administration of FGF and CXCL12 was able to nearly normalise platelet...
counts of thrombopenic thrombopoietin receptor-deficient mice, suggesting that CXL12-mediated interaction of progenitors with the BM vascular niche promoted their relocation to a microenvironment driving megakaryocyte maturation and thrombopoiesis (45).

Thus, platelet chemokines appear to be part of a control system balancing the platelet count by regulating megakaryocyte maturation and thrombopoiesis.

**Thrombosis and haemostasis**

Due to the high affinity of CXCL4 to negatively charged heparin and related GAGs, charge-dependent ultra-large complexes, can be formed. In patients exposed to heparin, antibodies against the CXCL4 tetramers/heparin complexes can occur which may lead to platelet activation by binding and clustering the receptor FcγRIIa increasing the risk for arterial and/or venous thrombosis, a syndrome termed heparin-induced thrombocytopenia (HIT) (46). Of interest, it is believed that the following thrombocytopenia is rather due to an intravascular sequestration by platelet activation than phagocytosis through the reticuloendothelial system (47). Patients diagnosed with HIT, who require anticoagulation, need an alternative to heparin. Interestingly, small molecule antagonists have been discovered and just recently used to disrupt CXCL4 tetramers thereby preventing the formation of the highly immunogenic macromolecular complexes with heparin which might represent a novel therapeutic approach (48).

Further evidence supported an important physiologic role of CXCL4 in haemostasis using CXCL4 knock-out and CXCL4-overexpressing mice (49, 50). Decreased thrombus formation was observed in a carotid artery injury model dependent on CXCL4 plasma levels. Concentrations exceeding a two-fold reduction or increase in CXCL4 were associated with impaired thrombus

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**Figure 1: Overview of the role of platelet-derived chemokines.** Platelets get activated by various stimuli followed by a release of α-granule chemokines. These biologically active mediators and their influence on specific (patho)physiological conditions, discussed in this review, are illustrated and ordered according to their assumed quantitative expression in platelets.
Platelet chemokines in infectious diseases

Antimicrobial activity in bacterial infections

Human platelets contain a number of antibacterial proteins in their α-granules which are released upon activation. Whereas chemokines that exert direct antimicrobial activity have been termed kinocidins, those originating from platelets are also known as thrombocidins (53). Characterisation of these proteins revealed that they are C-terminal truncation variants of the platelet-derived chemokines CXCL7, CXCL4 and CCL5 (54, 55). Altogether thrombocidins were found to display a broad spectrum of antimicrobial activity, more potent against *Escherichia coli*, *Staphylococcus aureus* than against the fungi *Candida albicans* and *Cryptococcus neoformans*. Moreover, inhibition of platelet microbicidal proteins aggravated bacteria-induced infective endocarditis (IE) in mice (56). In addition to direct antibacterial effects, thrombocidins indirectly strengthen the host defence by attracting leukocytes to the site of infection. However, the prognostic relevance of platelet chemokines and their derived microbicidal proteins for infections in humans is not clear.

Furthermore, bacteria can induce platelet activation by direct or indirect interactions. Dependent on the bacteria strain, distinct molecules are involved (57). For instance *S. aureus* rapidly activated platelets involving fibrinogen/fibronectin bridges between a bacterial binding protein such as clumping factor A or fibronectin-binding protein A (FnBPA) and resting integrin glycoprotein (GP)IIb/IIa, and specific immunoglobulin G (IgG) which engaged FcyRIIa, the ultimate receptor transducing the activating signal. Slow activation involved IgG via a platelet complement receptor (CR). Platelets express various functional Toll-like receptors (TLR) that led to platelet activation via TLR4 after challenge with gram negative bacteria or LPS (lipopolysaccharide). These platelets were able to activate neutrophils which released as a consequence DNA in form of extracellular traps (NETs) that in turn bound and inactivated bacteria (58, 59). Platelet-induced NETs were found as well to be relevant in transfusion-related or acute lung injury (TRALI/ALI) (60, 61). In ALI, platelet-derived CXCL4 and CCL5 formed leukocyte attracting heteromer complexes in the lung which was suggested to be due to a charge effect, neutralizing GAGs, allowing a closer contact between platelets and ECs. Additionally, CXCL4 promoted the thrombin-mediated generation of activated protein C (APC) by bridging thrombomodulin and the gamma-carboxyglutamic acid (Gla)-domain of protein C which improved survival during sepsis thereby interacting with thrombomodulin and Gla domain of APC (51, 52).

Malaria

Malaria is one of the most serious infectious diseases, caused by the parasite *Plasmodium falciparum*. Cerebral malaria is a complication by attachment of parasitized red blood cells to the endothelium in the brain, resulting in vascular inflammation, immune stimulation and obstruction of cerebral blood vessels (63). Platelet-derived CXCL4 plays a dual role during the pathogenesis of malaria, being beneficial in the early phase of infection and supporting the parasite elimination, whereas, at later stages, it sustains inflammation negatively influencing the course of disease. McMorran et al. showed that CXCL4 was released by platelets after CD36-mediated contact with parasitised red blood cells and a membrane protein of *P. falciparum*. Subsequently, CXCL4 translocated into the red blood cell and killed the parasite (64). In later stages, platelets deteriorated experimental cerebral malaria (ECM) through CXCL4-induced immune activation and T cell trafficking, thereby increasing ECM-associated inflammation (65, 66).

To develop novel drugs against malaria based on these new insights requires a better understanding about the CXCL4-dependent mechanism of parasite neutralisation.

HIV (human immunodeficiency virus)

Platelets and viruses have a bivalent relationship as exemplified by the infection with HIV involving platelet chemokines, predominantly CXCL4 and CCL5 (67). First, platelets were shown to bind and take up HIV via specific receptors (CLEC2, DC-SIGN), which may lead to spreading of the virus throughout the body via the circulation and bringing the virus into close contact with potentially virustatic platelet chemokines (68, 69). Interacting with its envelope protein gp120, HIV used CCR5 and/or CXCR4 as sequential co-receptors, in addition to CD4, to infect monocytes and lymphocytes, leading to a new HIV classification into R5, X4 and R5X4 variants (70). While CCL5, CCL3 and CCL4 were found to be major HIV-suppressive factors produced by CD8+ T cells (71), at least for CCL5 the opposite may be true since CCL5 was shown to enhance HIV infectivity by linking HIV via proteoglycans to the host cell (72). Chemokine-mediated control of HIV may either occur directly, through their inherent anti-retroviral activity, or indirectly by the attraction of immune cells. An additional explanation might be that the occupation of CCR5 by endogenous ligands may prevent HIV binding and cell entry.

All of the above mentioned chemokines have been identified to be expressed in platelets (see Table 1). Of interest, HIV infection was implicated in platelet activation resulting in a significantly higher CCL5 plasma concentration compared to healthy controls, implying platelets as an important source of CCL5 (73). In fact, the CCL5 receptor CCR5 can be targeted by small molecule antagonists, for instance maraviroc which is already clinically in use.
Notably, the role of anti-retroviral CXCL4 in the HIV pathophysiology has not been recognised for a long time. Only a recent study has identified CXCL4 as an endogenous antiviral factor, which inhibited infection of CD4+ T cells and macrophages by different phenotypic variants of HIV-1. CXCL4 blocked an early step in the HIV-1 infectious cycle at the level of virus attachment and entry via unique mechanisms that involved direct interaction with the major HIV-1 envelope glycoprotein, gp120 (74).

The identification of platelet-derived chemokines as endogenous antiviral factors may play an important role in the investigation of the pathogenesis and treatment of HIV-1 infection.

Atherogenesis and related vascular diseases

Atherosclerosis is an inflammatory disease of the arterial wall, during which platelet-induced chronic inflammation is a key step in atherogenesis and promotes plaque development, intimal hyperplasia and restenosis (75, 76). A decade ago, Huo et al. confirmed the crucial role of circulating activated platelets and platelet–monocyte complexes in promoting atherogenesis (77). Interactions of activated platelets with monocytes and atherosclerotic arteries led to the delivery of platelet-derived CCL5 and CXCL4 to the monocyte surface and endothelium of inflamed and atherosclerotic arteries triggering monocyte recruitment (77, 78). The genetic deficiency of CCL5 and CXCL4 has been found to reduce plaque formation in mice (79, 80), most probably mediated by decreased leukocyte recruitment into the arterial wall. However, platelet-derived chemokines may alter the immune system, for instance by inducing the differentiation of T cells and monocytes/macrophages (81-83). Also, CXCL4 formed heterodimers with CCL5, thereby synergistically enhancing the CCL5-mediated monocyte arrest (84). Remarkably, disrupting these interactions with a designed synthetic peptide in vivo resulted in decreased atherosclerotic lesion formation (80). Another possible mechanism responsible for the progression of atherosclerosis is the ability of CXCL4 to prevent monocyte apoptosis and to induce monocyte differentiation into macrophages (85). Furthermore, CXCL4 was found to inhibit the binding and degradation of LDL through its receptor, a process that could promote the formation of oxidised LDL (ox-LDL). In addition, CXCL4 directly bound to ox-LDL, increasing its binding to vascular cells and macrophages and enhanced esterification of oxLDL in macrophages. Also, CXCL4 co-localised with oxLDL in macrophage-derived foam cells of atherosclerotic lesions, a mechanism, that mediated vascular lipid accumulation (86). Accordingly, CCL5 secreted from activated platelets was found to be immobilised on the luminal surface of early atherosclerotic lesions after wire-induced injury or cytokine exposure, triggering the adhesion and transmigration of monocytes (78). Interestingly, a recent study indicated the role of CCL5 in cardiac infarction. Blocking CCL5 resulted in a smaller infarct size, reduced circulating levels of chemokines and decreased neutrophil and macrophage infiltration within the infarcted myocardium, improving mouse survival and cardiac function (87).

In addition, CXCL12, CXCL7 and CXCL7-derived peptides, which are released from platelets, were found to be relevant for the pathogenesis of vascular disease. As such, the early recruitment of EPCs to sites of arterial injury was mainly mediated by platelet-derived CXCL12 and CXCL7, requiring CXCR2 and CXCR4 (17, 88). Notably, plasma levels of CXCL7/NAP-2 were found to be increased in patients with stable angina pectoris compared to healthy control and even more pronounced in unstable angina pectoris. Furthermore, CXCL7/NAP-2 was detectable in macrophages and SMCs of atherosclerotic plaques and in platelets of coronary thrombi (89).

In line with these observations, SMC- and platelet-associated CXCL12, via CXCR4, has been demonstrated to mediate the preferential mobilisation and recruitment of a BM-derived SPCs subset from the circulation in response to arterial injury and apoptosis, thereby affecting neointimal hyperplasia (90-92).

Thus, platelet-derived chemokines may accelerate wound healing and regeneration and at the same time provoke atherogenesis, excessive cell proliferation and recruitment leading to arterial stenosis.

Conclusion

Platelets evolved from universal blood cells, as still present in nucleated thrombocytes from the horseshoe crab or the zebrafish that are in task of defending the organism against microbial pathogens and at the same time responsible for primary haemostasis, to highly specialised anucleate cells. It is conceivable that the production of dispensable proteins is ineffective and energy consuming being a disadvantage for the cell and organism. Hence, such superfluous molecules often get lost during evolution. Chemokines are evolutionary conserved proteins and some members are highly and selectively expressed in platelets. Surprisingly, deficiency of CXCL4 which is one of the highest expressed proteins in platelets does not cause an overt phenotype in mice. However, as summarised in this review, platelet chemokines can play various roles in health and disease influencing acute and/or homeostatic processes (Figure 1). Platelets are the first cells arriving at the site of injury, concomitantly stopping bleeding and attacking the invading pathogens. The released CXCL4 and CXCL7 truncation products (thrombocidins) exert antimicrobial activity. Intriguingly, excessive CXCL4 secretion is associated with impaired thrombosis/haemostasis.

Platelet-derived chemokines are important regulators of homeostasis and chronic diseases such as atherogenesis and neoplasia. CXCL4 provides antiproliferative cues that inhibit exceeding thrombogenesis, angiogenesis and stimulate differentiation, contrasted by CXCL12 which increases thrombogenesis. Based on the novel finding of morphologically diverse and compartmentalised α-granules containing distinct cargo, several hypotheses explaining the role of the concomitant storage of antagonistic molecules have been proposed. One concept suggests that coexisting thematically opposed mediators are stored in separate granules and are released through different signalling pathways enabling selec-
tive secretion. On the other hand, it has been suggested that the mediators are stochastically distributed to granules and their release is timed using different kinetics preventing unbalanced activity (93).

The recent gain of insights in the field of platelet-derived chemokines allows us to perceive upcoming therapeutic possibilities. Malaria for instance, is an enormous worldwide health issue that could be tackled by mimicking the CXCL4-based parasite neutralising mechanism. Moreover, one could envision controlling selective mediator release from platelets which would enable us to design strategies specifically addressing the underlying pathology. As an example, selectively inhibiting CXCL4 and CCL5 release would prevent the formation of the proatherogenic heteromer complexes. In addition, therapeutically disrupting the oligomerisation of platelet-derived chemokines may not only prove advantageous in the treatment of chronic inflammatory disorders such as atherogenesis but as in the case of disrupting CXCL4-tetramers may also be useful to treat HIT-related thromboembolic disorders. However, despite all of the intriguing findings emphasising the role of platelet-derived chemokine in (patho)physiology, it will take time to identify the most promising targets for translation into clinically applicable therapeutics.

Conflicts of interest
None declared.

References


