Platelets as Immune Cells: Bridging Inflammation and Cardiovascular Disease

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Abstract—Beyond an eminent role in hemostasis and thrombosis, platelets are characterized by expert functions in assisting and modulating inflammatory reactions and immune responses. This is achieved by the regulated expression of adhesive and immune receptors on the platelet surface and by the release of a multitude of secretory products including inflammatory mediators and cytokines, which can mediate the interaction with leukocytes and enhance their recruitment.

In addition, platelets are characterized by an enormous surface area and open canalicular system, which in concert with specialized recognition receptors may contribute to the engulfment of serum components, antigens, and pathogens. Platelet-dependent increases in leukocyte adhesion may not only account for an exacerbation of atherosclerosis, for arterial repair processes, but also for lymphocyte trafficking during adaptive immunity and host defense. This review compiles a selection of platelet-derived tools for bridging inflammation and vascular disease and highlights the molecular key components governing platelet-mediated mechanisms operative in immune surveillance, vascular remodeling, and atherosclerosis. (Circ Res. 2007;100:27-40.)

Key Words: platelets ■ inflammation ■ cardiovascular disease ■ chemokines ■ adhesion molecules

Undoubtedly, blood platelets play an eminent role in hemostasis and thrombosis. However, their distinctive armament with proinflammatory mediators as well as the presence of surface receptors predominantly known for their involvement in inflammatory or immune processes clearly exceeds an exclusive function as mere players in these primary physiological processes. Platelets amount to measures of roughly 300 000 cells per microliter of blood and individually feature a cell volume of 7 fL and a mean surface area of 8 μm². In aggregate, blood platelets thus display a larger total volume and surface area than the aggregate of all other leukocyte subtypes taken together. On activation, platelets exhibit the ability to release considerable quantities of secretory products and express a multitude of immune receptors on their membrane. In addition, platelets are characterized by an open canalicular system, which may contribute to the engulfment (and/or “filtration”) of serum components, pathogens, or antigens. As protagonists (and more than supporting actors) in both inflammatory and immune processes reviewed herein, platelets represent an ideal and crucial link to explain the inseparability of thrombotic and inflammatory events, as it occurs in cardiovascular diseases such as atherosclerosis or atherothrombosis. This review summarizes the most important findings on the role of...
platelets according to their molecular key constituents with immune and/or inflammatory relevance, laying out a "toolbox" for the participation of platelets as immune cells, and gives an update of the intensively investigated involvement of platelets in inflammation and the immune system.

**Surface Molecules Linking Platelets to the Immune System**

**P-Selectin**

P-Selectin is an integral membrane glycoprotein expressed by platelets\(^1\) and endothelial cells\(^2\) but has also been recently found in macrophages of atherosclerotic plaques\(^3\) (Figure 1). Cellular activation leads to rapid redistribution from the secretory \(\alpha\)-granules of platelets and Weibel–Palade bodies of endothelial cells to the plasma membrane.\(^4\) By binding its major ligand P-selectin glycoprotein ligand-1 (PSGL-1), P-selectin initiates the adhesion of platelets by establishing reversible bonds that transform tethering into rolling and subsequently allow firm arrest. Intravital microscopy in P-selectin−deficient and wild-type mice revealed that regardless of their stimulation platelets roll on activated endothelium in vivo, which requires endothelial but not platelet P-selectin.\(^5,6\) However, P-selectin on platelets is an important adhesion molecule for PSGL-1−bearing immune cells, as it mediates adhesion of activated platelets to monocytes, neutrophils, and lymphocytes, resulting in the formation of platelet/leukocyte complexes and, vice versa, supports leukocyte rolling and arrest on surface-adherent platelets.\(^7–9\) In vitro, activated platelets enhance tethering of lymphocytes to PNAd (peripheral lymph node addressin) and sustain lymphocyte rolling, even in the absence of functional L-selectin. In an animal model, platelets facilitate lymphocyte delivery to high endothelial venules (HEVs) by platelet P-selectin, which allows activated platelets to transiently form a bridge between lymphocytes and HEVs, thereby enabling lymphocytes to undergo subsequent \(\beta_2\) integrin−dependent firm adhesion.\(^7,10\) This alternative adhesion pathway is sufficient to reestablish lymphocyte trafficking in L-selectin−deficient mice and restores a contact hypersensitivity immune response to cutaneous antigen. In a similar fashion, crosslinking of PSGL-1 on monocytes by platelet P-selectin induces upregulation and activation of \(\beta_1\) and \(\beta_2\) integrins and enhances monocyte recruitment to activated endothelium.\(^11\) Furthermore, most monocytes bind soluble platelet-derived P-selectin, which does not alter the expression of the \(\alpha\)\(\beta_1\) integrin VLA-4 (Very-Late Antigen-4) on monocytes but enhances its adhesiveness. It has been inferred that PSGL-1/P-selectin interactions thereby promote the binding of VLA-4 to vascular cell adhesion molecule-1 (VCAM-1) during the sequential adhesion cascade that regulates monocyte trafficking to inflammatory and atherosclerotic lesion.\(^12\)

The CC chemokine RANTES released by stimulated platelets can be immobilized and presented on activated endothelium, where it enhances monocyte recruitment\(^13\) (Figures 2 and 3). The finding that endothelial deposition of RANTES by platelets is more effective in flow than in stasis led to the discovery that platelet P-selectin but not PSGL-1 was critical for the deposition of platelet-derived RANTES as a prerequisite for its arrest function on activated endothelium.\(^14\) Platelet activation encompasses multiple cellular responses, including the formation of platelet microparticles (Figure 3). The concept that RANTES deposition is supported by transient platelet microparticle interactions with endothelium may invoke a role of adhesion and/or signaling receptors in

![Figure 1. Platelet adhesion molecules and surface receptors. For clarity, the symbols for the various protein domains depicted are explained within the Figure.](http://circres.ahajournals.org/)}
the delivery of RANTES. The diffuse distribution pattern of RANTES, which was not significantly colocalized with firmly adherent platelet microparticles, suggests a more important role of molecules inducing transient interactions rather than firm arrest. Indeed, blocking P-selectin but also GPIb or junctional adhesion molecule (JAM)-A reduced deposition of platelet-derived RANTES and subsequent monocyte arrest.15 Although chemokines presented on the endothelium trigger integrin activation, an additional mechanism has been proposed, by which P-selectin–mediated signaling directly activates integrin clustering. It has been proposed that crosslinking PSGL-1 induces clustering of lymphocyte function-associated antigen-1 (LFA-1) on Th1 cells16 and clustering of Mac-1 on neutrophils.17

In conjunction, these findings support the notion that P-selectin on platelets is crucial for the recruitment of immune cells by dual action as an adhesion and signaling molecule. It enables the close contact of platelets with inflammatory blood-borne cells and induces rolling of these cells that together with arrest chemokines entails the activation of integrins important in mediating firm adhesion. Furthermore, platelet P-selectin is instrumental in facilitating the delivery and immobilization of platelet-derived chemokines on activated or atherosclerotic endothelium as culprits for mononuclear cell infiltration. It will be subject to further investigation how ligands for P-selectin expressed on endothelial cells, possibly PSGL-1, and their modification under inflammatory conditions may contribute to these processes.

**Integrins**

Platelets express several integrins (Figure 1), eg, α₄β₁, α₅β₁, α₅β₃, or α₆β₃, but α₅β₃ (glycoprotein GPIIb/IIIa) is clearly the predominant one18,19 and has recently been found to be functionally active in mast cells and hematopoietic progenitors.20 By forming a bridge to adhesive proteins, such as fibrinogen, von Willebrand factor, or fibronectin, α₅β₃ mediates arrest of activated platelets to adhesion molecules intercellular adhesion molecule (ICAM)-1 and α₅β₁ on endothelium (Figure 3), as indicated by in vitro studies.21 Integrins form an interface between extracellular membrane proteins and intracellular proteins, enabling bidirectional information transfer. Ligation of α₅β₃, through multivalent ligands, eg, fibrinogen, leads to clustering and outside-in signaling, which is important in cytoskeletal rearrangement in the process of aggregation.22,23 The binding of the cytoplasmic tails of both subunits keeps α₅β₃ in a resting state. Activation of platelets results in binding of the key regulator talin to the β tail that in turn results in splaying of the α and β tails, and triggers an inside-out conformational signal for receptor activation and exposure of its ligand-binding pocket.24,25 Integrin α₅β₃ links platelets to sites of injury in vessels through interaction with fibrinogen and von Willebrand factor, which in aggregate form a plug leading to hemostasis.

Another binding partner of α₅β₃ has been shown to be CD40 ligand (CD40L) expressed on the membrane of activated platelets, which is important in stabilizing arterial thrombi.26 The KGD domain of sCD40L binds to α₅β₃, triggering outside-in signaling by tyrosine phosphorylation of β₃.27 Thus, CD40L binds to α₅β₃ in thrombosis and inflammation. Blocking α₅β₃ by intravenous antagonists reduces the rise in inflammatory markers, such as C-reactive protein and interleukin (IL)-6, after coronary interventions and the antiinflammatory effects of this antagonism have been held responsible for long-term clinical benefits.28 Furthermore, a relation of α₅β₃ to inflammation can be deduced by the...
finding that specific types of \( \alpha_{ii} \beta_{3} \) inhibitors can increase the release of platelet \( \alpha \) granules, despite inhibiting agonist-induced fibrinogen binding.\(^{29}\) By binding and possibly stabilizing, the active conformation of \( \alpha_{ii} \beta_{3} \) antagonists may be proinflammatory at suboptimal doses, but, by blocking platelet aggregation, they may inhibit inflammation and thrombosis, as is evident by reduced release of inflammatory mediators sCD40L (soluble CD40L) or RANTES.\(^{30,31}\) However, this may be particularly relevant under conditions of arterial injury, eg, after percutaneous intervention, with abundant platelet activation, whereas transient interactions of platelets may be sufficient during atherogenesis.

**CD40 Ligand**

Besides activating platelet \( \alpha_{ii} \beta_{3} \), CD40L (also termed CD154) plays a crucial role in pathogen clearance. As a trimeric transmembrane protein (Figure 1), it is structurally related to the cytokine tumor necrosis factor (TNF)-\( \alpha \) and was originally identified on stimulated T cells.\(^{32,33}\) Subsequently, platelets were found to upregulate CD40L on activation and aggregation via arachidonic acid-mediated gp91phox activation, which results in stimulation of endothelial cells through its cognate receptor CD40 and in increased expression of adhesion molecules and chemokines enhancing the recruitment of immune cells.\(^{34,35}\) These results prompted investigations that demonstrated the link of platelet-derived CD40L and atherosclerosis.\(^{36–39}\) The role of platelets has also been extended beyond inflammatory reactions or innate immunity. Ligation of CD40 by platelet-derived CD40L induces dendritic cell maturation, B-cell isotype switching, and augmentation of CD8\(^{+} \) T cell responses both in vitro and in vivo, causing enhanced protection against viral challenge.\(^{40}\) In contrast, depletion of platelets in wild-type mice results in decreased antigen-specific antibody production.\(^{40}\) Together with findings that platelets alone or low amounts of CD4\(^{+} \) T cells per se are not sufficient in the formation of germinal centers, although a combination of both is sufficient, led to the notion that platelets exert a sentinel function, enhancing signals required for robust adaptive humoral immunity.\(^{41}\) Platelet-derived or even soluble CD154 has recently been found to be sufficient for the initiation and induction of vascularized allograft (eg, cardiac) rejection independent of cell-bound sources of this molecule.\(^{42}\) Platelet-derived sCD40L is also elevated and biologically active in sickle cell anemia. The participation of sCD40L in sickle cell anemia plasma–induced production of B cells, tissue factor, and ICAM-1 suggests that CD40L may contribute to the chronic inflammation and increased thrombotic activity known to occur in this disorder.\(^{43}\)

**CD40**

Serving as the receptor for CD154 (CD40L), CD40 is a trimeric transmembrane protein and belongs to the TNF receptor superfamily (Figure 1). CD40 is displayed by a wide range of immune cells including monocytes, dendritic cells, and B cells and contributes to the development of the acquired immune response, depending on its activation and subsequent signal transduction by CD40L.\(^{44}\) CD40 is not only constitutively expressed on platelets but plays an important role in confining the inflammatory response induced by CD40L. This occurs by inducing the cleavage of CD40L, which results in a 18-kDa soluble form without an inflammatory effect on endothelial cells.\(^{37,45}\) In contrast, sCD40L retains its ability to stimulate platelets through CD40. Similarly, T-cell bound CD40L can activate platelets and triggers the release of RANTES, which is followed by enhanced T-cell recruitment, epitomizing a proinflammatory feedback loop.\(^{38}\) In addition, sCD40L can enhance platelet aggregation, and platelet-leukocyte conjugation, and can potentiate agonist-induced release of reactive oxygen intermediates by platelets.\(^{46}\) Whether platelet CD40 or CD40L prevail in promoting vascular inflammation remains to be determined using bone marrow chimeras or platelet-specific deletion.

**Intercellular Adhesion Molecule 2**

ICAM-2 is a 55-kDa member of the immunoglobulin (Ig) superfamily of adhesion molecules, composed of 2 N-terminal Ig domains with 35% homology to ICAM-1, a transmembrane domain, and a short cytoplasmic tail. As a counter-receptor for the leukocyte \( \beta_{2} \) integrin LFA-1 (\( \alpha_{II} \beta_{3} \), CD11a/CD18) and for the dendritic cell-specific, ICAM-grabbing nonintegrin (DC-SIGN), ICAM-2 is constitutively expressed on endothelial cells, most leukocytes, and platelets. Also expressed on platelets, DC-SIGN may participate to the engulfment of pathogens into platelets. Because ICAM-1 and ICAM-3 were not detectable, ICAM-2 was thought to be the principle \( \beta_{2} \)-integrin ligand present on platelets. Platelet-derived ICAM-2 is not upregulated on activation and has been shown to mediate lymphocyte-platelet adhesion via LFA-1 (or DC-SIGN) and may also contribute to the transition of neutrophil rolling into Mac-1–dependent firm arrest under flow conditions.\(^{47–49}\) Notably, postranslational modification of ICAM-2 by physiologic sialylation renders it less able than endothelial ICAM-2 to support adherence of leukocytes.\(^{50}\)

**Junctional Adhesion Molecules**

The F11 receptor (F11R, or JAM-A) was originally identified on platelets by the ability of the monoclonal antibody F11 to induce \( \alpha \)-granule release and shape change, which was attributable to crosslinking of the antigen with Fc\(\gamma\)RII.\(^{51}\) Subsequent studies revealed that crosslinking of F11R without Fc\(\gamma\)RII causes signal transduction by protein kinase C, adhesion, and formation of homodimers.\(^{52}\) The cDNA clone for this molecule was found in 1998, encoding a protein present within tight junctions of endothelial cells and termed junctional adhesion molecule-A (JAM-A).\(^{53}\) As the prototypic member of the JAM family, which forms a subclass within the Ig superfamily (Figure 1), JAM-A predominantly occurs as a homodimer.\(^{54,55}\) Trans interactions of platelet-derived JAM-A have been found to support the luminal deposition of platelet chemokines and to enhance the recruitment of leukocytes.\(^{15,56}\) Heterophilic binding of JAM-A by the integrin LFA-1 and its role in leukocyte transmigration in vitro and in vivo implies similar physiological functions during platelet/leukocyte interactions. By analogy, a third member of this family, JAM-C, was identified as a novel counter-receptor on platelets for the leukocyte \( \beta_{2} \) integrin
Mac-1 (α3β2, CD11b/CD18) mediating platelet-neutrophil interactions.69 In concert with the binding of Mac-1 to GPIb or fibrinogen presented by GPIb/IIa,48,60 this may particularly relevant for an inflammatory role of platelets in vascular injury and atherothrombosis. The relative and nonredundant contribution of different Ig adhesion molecules, in particular of JAMs, to the interactions of platelets with leukocytes and endothelial cells needs to be further clarified in detail.

**Toll-Like Receptors**

The human body is permanently challenged by different pathogens such as bacteria, viruses, fungi, and parasites. The initial clearance by innate immune responses is mediated by a group of receptors termed Toll-like receptors (TLRs). These type I integral membrane proteins (Figure 1) recognize common pathogen-associated molecular patterns and are characterized by an extracellular domain containing multiple leucine-rich repeats, a single transmembrane domain, and an intracellular Toll/IL-1 receptor domain, which on activation gathers multiple adaptor proteins and transduces signals.61 Typical ligands of TLRs comprise bacterial wall components, eg, lipopolysaccharide (LPS), which binds and activates TLR4 or nucleic acids. Intriguingly, TLRs can recognize structurally different molecules, as is evident by TLR4, which does not only serve as the receptor for LPS but also binds to paclitaxel, heat-shock proteins and fibrinogen with high affinity.61 TLRs are expressed by a variety of immune cells and some subtypes have recently been discovered on resting platelets and detected by immunohistochemistry of human coronary thrombi, suggesting a link between infectious immunity and arterial thrombosis.52 At least for TLR4, functional implications have been demonstrated in sepsis models. Similar to septicemia in humans, LPS has been shown to induce thrombocytopenia in a mouse model through TLR4-dependent sequestration of platelets to the lungs. This has been attributed to platelet activation via TLR4, which resulted in increased expression of P-selectin, in line with the inability of LPS to induce platelet aggregation or calcium influx.62,63 Under arterial flow conditions, LPS-stimulated platelets did not induce thrombocytes, whereas platelet activation was seen in LPS-stimulated platelets when washed platelets were used. Providing a hint that the presence of chemokine receptors alone may not be sufficient for biological activity in platelets.75,77 Because platelets in plasma in turn react on SDF-1, primarily because of decreasing cAMP levels, plasma components appeared to be required for platelet activation. Moreover, it has become apparent that intracellular signaling through CXCR4 changes during cell differentiation, eg, of B cells,79 and is affected by the differentiation-dependent expression of proteins interfering with signal transduction, eg, “regulators of G-protein signaling” (RGS). Indeed, RGS16 is upregulated during maturation of megakaryocytes and can inhibit SDF-1 signaling.80 Eventually, several studies have revisited platelet aggregation experiments with SDF-1 as a stimulus, demonstrating calcium influx.81–83 Under arterial flow conditions, SDF-1 induced weak aggregation that was strongly dependent on the presence of low amounts of the primary agonists ADP or thrombin that per se had only a slight effect.81 The same result could be obtained by stimulating platelets in plasma, in this case suggesting a role of low amounts of platelet agonists.78 A role for this mechanism may be envisioned during the development of atherosclerotic plaques, where SDF-1 is markedly detectable and could lead to platelet activation and formation of platelet-rich thrombi.83 Alternatively, SDF-1 may mediate the recruitment of circulating progenitors to support vascular regeneration, thrombus resolution, or remodeling.84,85 Independently, the functional expression of the chemokine receptors CCR1, CCR3, and CCR4 has been shown in platelets (Table 1). CCR4 is a receptor for MDC (CCL22) and TARC (CCL17). A comprehensive study showed calcium influx in platelets stimulated by TARC and other chemokines, as well as synergistic aggregation with

**Chemokine Receptors**

Chemokine receptors are 7-transmembrane proteins (Figure 1) that are coupled to G-protein heterotrimers and initiate signal transduction events leading to a multitude of cellular responses, in particular chemotaxis and adhesion.58–70 Some chemokine ligand/receptor pairs have been critically involved in both organ development and immune homeostasis. For instance, the targeted disruption of CXCR4 (to date, the only receptor for stromal cell–derived factor-1 [SDF-1/CXCL12]) resulted in embryonic lethality, with severe defects in hematopoiesis and vasculo- and cardiogenesis that closely resembled those seen in SDF-1–deficient mice, indicating that CXCR4 may be the major receptor for SDF-1.71–73 As insinuated by its universal expression pattern, the first evidence for the functional presence and membrane display of chemokine receptors on platelets was also provided for CXCR4. In vitro studies revealed its existence on the megakaryocytic lineage from progenitors to platelets and demonstrated that its ligand SDF-1 is able to trigger adhesion of megakaryocytes on endothelium and to induce migration of megakaryocyte precursors.74–77 Interestingly, although flow cytometry clearly confirmed the presence of CXCR4 on platelets, neither calcium influx nor upregulation of P-selectin and platelet aggregation occurred when washed platelets were used, providing a hint that the presence of chemokine receptors alone may not be sufficient for biological activity in platelets.75,77 Because platelets in plasma in turn react on stimulation with SDF-1, primarily because of decreasing cAMP levels,78 plasma components appeared to be required for platelet activation. Moreover, it has become apparent that intracellular signaling through CXCR4 changes during cell differentiation, eg, of B cells,79 and is affected by the differentiation-dependent expression of proteins interfering with signal transduction, eg, “regulators of G-protein signaling” (RGS). Indeed, RGS16 is upregulated during maturation of megakaryocytes and can inhibit SDF-1 signaling.80 Eventually, several studies have revisited platelet aggregation experiments with SDF-1 as a stimulus, demonstrating calcium influx.81–83 Under arterial flow conditions, SDF-1 induced weak aggregation that was strongly dependent on the presence of low amounts of the primary agonists ADP or thrombin that per se had only a slight effect.81 The same result could be obtained by stimulating platelets in plasma, in this case suggesting a role of low amounts of platelet agonists.78 A role for this mechanism may be envisioned during the development of atherosclerotic plaques, where SDF-1 is markedly detectable and could lead to platelet activation and formation of platelet-rich thrombi.83 Alternatively, SDF-1 may mediate the recruitment of circulating progenitors to support vascular regeneration, thrombus resolution, or remodeling.84,85 Independently, the functional expression of the chemokine receptors CCR1, CCR3, and CCR4 has been shown in platelets (Table 1). CCR4 is a receptor for MDC (CCL22) and TARC (CCL17). A comprehensive study showed calcium influx in platelets stimulated by TARC and other chemokines, as well as synergistic aggregation with
TARC, MDC, RANTES (CCL5), and SDF-1 (Tables 1 and 2). Accordingly, findings that the chemokines TARC, MDC, and SDF-1 alone are not sufficient to induce platelet aggregation but need low levels of platelet agonists such as ADP or thrombin may explain such differences. Similar to the SDF-1/CXCR4 axis, CX3CR1, the receptor for fractalkine (CX3CL1) is functionally expressed on platelets and, independently of the presence of ADP, triggers G$_i$ activation but not calcium influx, and triggers P-selectin upregulation and platelet adhesion to collagen and fibrinogen. Fractalkine may substantially contribute to platelet activation under conditions of vascular diseases, eg, vascular remodeling or atherosclerosis, where this chemokine is upregulated. Consistently, disruption of CX3CR1 in atherosclerosis prone mice results in a reduced plaque formation and a more stable plaque phenotype. However, the cell-specific role, relevance, and hierarchy of CX3CR1 and other chemokine receptors in platelets for activating or potentiating their immune functions will be further dissected by selective deletion in platelets.

### TABLE 1. Expression and Function of Platelet Chemokine Receptors

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<th>mRNA</th>
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<th>Aggregation</th>
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ND indicates not determined; hu, human; mu, murine; r, rat.

### TABLE 2. Platelet-Derived Chemokines

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<th>Alias</th>
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<th>Receptor</th>
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ND indicates not determined; hu, human; mu, murine.
Soluble Immune Mediators

Chemokines

Among the abundance of platelet-derived secretory products with inflammatory properties (Figure 2), the first member of the chemokine family was discovered in releases from platelets and was named platelet factor 4 (PF4, CXCL4). The term chemokine was eponymously defined by their prominent feature as chemotactic cytokines, and coined approximately 20 years later by the identification of IL-8. Since then, a large family of more than 50 related proteins has evolved and has been classified according to their structure. Depending on the position of the first N-terminal cysteines that are either adjacent or separated by 1 amino acid residue, chemokines are divided into 2 large subfamilies (CC and CXC chemokines), with fractalkine (CX3CL1) displaying 3 amino acids to separate the cysteines and lymphotactin with 1 cysteine. In platelets, chemokines synthesized by megakaryocytes are stored within the α granules and may, in part, be expressed from mRNA transcripts that can be found in mature platelets. PF4 together with the β-thromboglobulins (β-TGs) are the most abundantly expressed platelet CXC chemokines and can be further divided by the absence (PF4) or presence (β-TG) of an N-terminal ELR-motif, which enables binding to and activation of the chemokine receptors CXCR1 and CXCR2. In turn, PF4 differs from most other chemokines in that it exerts its biological activity at much higher concentrations. Both a specific proteoglycan and the G protein–coupled receptor CXCR3B have been reported to be responsible for its actions. Although PF4 has been found in other cell types, it appears to be most relevant in platelets because of the most substantial expression levels achieved. In the presence of appropriate costimuli, such as TNF-α, PF4 induces exocytosis and firm neutrophil adhesion to endothelium. Additional biological characteristics include the inhibition of proliferation of many cell types, eg, endothelial cells, which may explain its angiostatic activity and myeloid progenitor cells. Like most chemokines, PF4 has a considerable affinity for glycosaminoglycans, eg, heparan sulfate, and interacts with other heparin-binding proteins. Notably, PF4 can block the activation of human progenitor cells by IL-8 because of its high affinity for IL-8. It appears that PF4 exerts its activity by interacting with these molecules resulting in differential effects that are not fully understood, eg, it blocks monocyte adhesion induced by IL-8, whereas it enhances monocyte adhesion induced by RANTES/CCL5.

All of these chemokines are secreted by stimulated platelets (Table 2). Depending on their concentrations and affinities, heterophilic interactions of these chemokines may occur in preformed complexes of platelet granules, integrating synergetic or antagonistic cues for monocyte arrest, as proposed by the “chemokine interactome” (Figure 3). Because mice do not express an IL-8 ortholog, it will be interesting to see whether this principle can also be applied to the closest murine relative GRO-α/CXCL1.

The CC chemokine RANTES has been found by using a cDNA library enriched for T cell–specific sequences and causes the selective migration of human blood monocytes and T cells. Later, thrombin stimulation of human platelets was shown to result in the release of a preformed peptidic eosinophil–chemotactic activity that could be further purified to RANTES. RANTES clearly contributes to vascular inflammation in the context of atherosclerosis and neointima formation after vascular injury. In vitro perfusion of endothelial cells with activated platelets, their supernatants or platelet-derived microparticles leads to P-selectin–dependent endothelial deposition of RANTES (Figure 3) and increased monocyte recruitment on activated or early atherosclerotic endothelium. This could be reproduced in vivo, where injection of activated platelets in atherosclerosis-prone mice leads to P-selectin–dependent deposition of PF4 and RANTES and exacerbates atherosclerosis, whereas inhibition of RANTES receptors results in decreased lesion size, both in atherosclerosis and vascular injury.

Furthermore a pivotal role of platelet-associated chemokine SDF-1 in vascular injury and remodeling has been established as immobilized platelets at the site of injury not only present SDF-1 thereby recruiting a subset of SMC progenitors but also secrete SDF-1 to support primary adhesion and migration of progenitor cells. As others have failed to detect a secretion of SDF-1 by human megakaryoblasts, the relevance of these findings in the human system needs to be confirmed. The group of platelet-derived chemokines with inflammatory and immune implications also includes TARC, which plays a crucial role in atopic dermatitis, because its release by platelets, attracting Th2 cells, correlates with disease activity. Collectively, platelets contain a multitude of chemokines that link platelet activation to the recruitment of immune cells by displaying or depositing chemokines on cell surfaces and activating their cognate receptors (Figure 2), resulting in enhanced integrin activity and thereby exacerbating vascular disease. The interference with immobilization sites or synergistic heteromerization of chemokines at sites of inflammation will be explored to provide novel avenues for therapeutic targeting.

Cytokines: Interleukin-1

Duplication of a common gene during evolution has produced three members of the IL-1 gene family: IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1RA). IL-1 is the prototypic cytokine released by inflammatory cells. Whereas IL-1α, because of the lack of a leader sequence, is primarily an intracellular regulator of proinflammatory events, its sibling IL-1β is a secretable mediator and the structurally related protein IL-1Ra is a naturally occurring antagonist competing for the signaling receptor IL-1R1. In vitro and in vivo experiments have shown multiple biological effects of IL-1 including increased production of chemokines and upregulation of endothelial adhesion molecules. The synthesis, processing, secretion, and activity, particularly of IL-1β, are tightly regulated processes. The administration of recombinant IL-1RA prevents inflammatory responses in animal models of inflammatory diseases and has been successful applied in rheumatoid arthritis. A role of IL-1β for atherosclerosis has been proposed for many years. The expression of IL-1β has been found to be increased in arteries of hyperlipidemic animals and in monocytes incubated with oxidized LDL, and in a transgenic animal model a high ratio of IL-1 to IL-1RA results in exacerbation of atherosclerotic
lesions.\textsuperscript{130–132} Besides monocytes, platelets have been identified as major participants in the complex inflammatory development of atherosclerotic lesions.\textsuperscript{133} IL-1 activity of platelets was demonstrated by findings that activated human platelets and their releasates led to reactions typically for IL-1 such as IL-1–dependent cell proliferation or upregulation of adhesion molecules and chemokine expression by endothelial cells (Figure 2) that in turn could be blocked by antibodies against IL-1 or IL-1 receptor antagonists.\textsuperscript{134–137} Subsequently, it was demonstrated that platelets do not only contain preformed IL-1β but that platelet activation induces rapid and sustained synthesis of pro-IL-1β protein, which is shed in its mature form in membrane microvesicles and is controlled at the translational level by activation of platelet integrins.\textsuperscript{138,139} Unexpectedly, platelets were found not only to regulate IL-1 translation but also to possess a functional spliceosome, a complex that processes pre-mRNAs in response to integrin engagement or surface receptor activation.\textsuperscript{140} These findings indicate a hitherto unrecognized role of platelets in vascular inflammation or injury by connecting platelet activation, cytokine expression, and inflammatory responses.

**Microbicidal Proteins**

Antibacterial proteins, such as defensins, are components of the innate immune system found in many organisms and produced by a variety of cell types\textsuperscript{141} and are most abundantly secreted by neutrophils, but epithelial tissues and human airway tissue among others are a rich source as well.\textsuperscript{142–144} In addition to these cell types, human and rabbit platelets are known to store antibacterial proteins.\textsuperscript{145–147} These antibacterial proteins (Figure 2) are released from platelet α granules after stimulation with thrombin and were designated thrombicidins\textsuperscript{148} or thrombin-induced platelet microbicidal proteins.\textsuperscript{149} Partial characterization revealed that the bioactive protein moiety in human platelets was cationic and of low molecular weight\textsuperscript{145,146} and possesses potent bactericidal activity against a significant portion of *Staphylococcus aureus* isolates. Further characterization revealed 2 thrombicidins (TC-1 and -2), which correspond to C-terminally truncated forms of NAP-2 and CTAP-III that, in contrast to the full-length forms, were bactericidal against some strains of *Bacillus subtilis*, *S. aureus*, and fungicidal for *Cryptococcus neoformans*.\textsuperscript{150} Additional human chemokines, or their related orthologs, that exhibit antimicrobial activity in vitro have been identified.\textsuperscript{151–153} In line with these in vitro studies, full-length IL-8 did not exhibit antimicrobial activity, but C-terminal peptides of IL-8 revealed activity against bacteria, demonstrating that the amphipathic α helix is the responsible structure.\textsuperscript{154} The cationic nature of these proteins is thought to be crucial to target and disrupt microbial membranes as they exhibit structural familiarity with cecropin-like peptides that affect their antimicrobial activity through formation of pore structures in phospholipid bilayers, leading to depolarization of the target membrane,\textsuperscript{151,155} but may additionally interfere with intracellular macromolecular synthesis.\textsuperscript{156} Because the concentrations that are needed for a relevant antimicrobial activity compared with that for inducing leukocyte migration are considerable, the question remains whether such concentrations can be achieved in vivo. This would have to be verified in relevant models of microbial infection. Among the CC as well as CXC chemokines with antibacterial activity, many are stored in platelets (SDF-1, GRO-α, TARC, etc.), providing an additional link for the in vivo relevance of platelets in coupling injury and inflammation.

**Platelets Are Active Players in Inflammatory and Immune Disease**

**Platelets in Infectious Disease**

Interactions of bacterial pathogens with platelets take place in variety of clinical situations, endocarditis and cardiovascular disease being among the most intensively studied.\textsuperscript{157} Platelets interact with Gram-positive, Gram-negative bacteria and spirochetes, respond rapidly in the setting of vascular injury, aggregate after contact with bacteria through crossreactive immunodeterminants and plasma proteins, and have the ability to recognize pathogens via TLRs.\textsuperscript{62,158,159} These interactions have a common mechanism: bacteria target host fibronectin, collagen, or fibrinogen by proteins, anchored in the bacterial cell wall such as fibronectin-binding proteins (FnBPs),\textsuperscript{160} clumping factor,\textsuperscript{161} and platelet aggregation–associated protein (PAAP),\textsuperscript{162} which creates a protein bridge with a platelet receptor, usually GPIIb/IIIa or GPIb. This interaction in itself is insufficient for platelet activation but requires a circulating antibody specific for the bacterial surface protein to engage the platelet FcγRIIa receptor,\textsuperscript{163,164} which induces platelet activation, aggregation, and thrombus formation. Because of these rapid responses, platelets accumulate in the setting of endovascular infections such as infectious endocarditis where platelets represent a significant portion of infected cardiac vegetations and emboli and have been traditionally interpreted as contributors to the progression and complications of infectious endocarditis.\textsuperscript{165,166} In an animal model for infectious endocarditis, the in vitro susceptibility to platelet derived antimicrobial peptides correlates well with disease severity and prognosis,\textsuperscript{146,167–169} but other mechanisms may be relevant for the critical role that platelets play for infections. Internalization of bacteria under resting conditions appears to be rare. When platelets are stimulated by agonists such as ADP or SDF-1, bacterial internalization is strongly increased and further enhanced under flow conditions.\textsuperscript{170,171} It remains controversial whether engulfment of the bacteria takes place within the open canalicular system, as suggested by staining with osmium black,\textsuperscript{172,173} or in separated vacuoles, as shown by the lack of typical protein composition of the vacuole membrane.

Besides bacteria, platelets are able to internalize HIV-1 and lentivirus\textsuperscript{170,174,175} through CLEC-2 (C-type lectin-like receptor 2) and DC-SIGN expressed on platelets.\textsuperscript{176} The capture of HIV-1 by these cells amounts to a considerable threat for the immune system, as platelets can remain infectious over a prolonged time, indicating that platelets might facilitate HIV-1 dissemination. How this can be targeted in an effort to reduce viral load is an open question to be solved by future studies.

**Atherosclerosis**

Platelets and monocytes are thought to play a predominant role in the initiation and progression of atherogenesis.\textsuperscript{133} According
to the many studies investigating the role of platelets in atherosclerosis, this disease involves many of the molecules outlined above, leading to platelet-facilitated migration of monocytes and other mononuclear cells into the arterial wall.

In different mouse models for atherosclerosis (LDL-receptor−/− or apolipoprotein E−/−), P-selectin deficiency has been found to be associated with decreased formation of fatty streaks, which represent early atherosclerotic lesions,177 but a reduction in lesion area has also been found in more advanced atherosclerotic plaques.178,179 Because P-selectin is predominantly displayed on platelets and endothelium, bone marrow transplantation of P-selectin−/− was used to dissect the role of both cell types and revealed a significant contribution of platelets besides endothelial cells.180 P-selectin–dependent adhesion of platelets to monocytes results in circulating complexes with increased adhesion to endothelial cells under coronary flow conditions.181 Thus, transient P-selectin or PSGL-1 blockade at the time of arterial injury significantly limits plaque macrophage content and neointima formation in a dose-dependent manner after endothelial denudation injury of carotid arteries in apolipoprotein E−/− mice.182

P-Selectin is among the adhesion molecules of platelets and platelet-derived microparticles leading to prolonged contact with endothelial cells and deposition of chemokines that activate and arrest monocytes and exacerbate atherosclerotic lesions in mice.13–15,111,119 Moreover the strong proinflammatory mediator IL-1 is secreted by platelets and has an impact on atherosclerosis that is mediated at least by inducing alterations of the adhesive and chemotactic properties of endothelial cells.130,137,183–185

The glycoproteins GPIb/IIa and GPIb participate in platelet adhesion to the endothelium of atherosclerosis-prone mice that have not yet developed plaques and are involved in the deposition of chemokines from platelet-derived microparticles.15,186 As underlying mechanisms, the involvement of GPIb may encompass binding to von Willebrand factor to support platelet adhesion or to leukocyte Mac-1 to support platelet-leukocyte aggregates.60,181 The CD40–CD40L dyad is well known to play an important role in atherogenesis especially in inducing a plaque phenotype with a rich inflammatory infiltrate which is unstable and more likely prone to rupture with subsequent atherothrombosis and infarction.187,188 However, a direct link of platelet-derived CD40 or CD40L has not been yet established. Indeed, although an elevation of soluble CD40L in plasma has been useful to identify patients with unstable coronary artery disease at risk for acute syndromes and likely to benefit from antiplatelet treatment,189 an elegant population-based study revealed that sCD40L was not associated with subclinical atherosclerosis or subclinical risk factors, but it was not determined whether sCD40L serves as a marker for future cardiac events.190 Thus, it is conceivable that sCD40L may be more suitable as a predictor of plaque vulnerability or acute syndromes with overt platelet activation.

In the context of vascular injury, platelets constitute repair modules that can cover sites of damages with exposed matrix and can present the necessary adhesive anchors and arrest chemokines to trigger the recruitment of circulating mononuclear and progenitor cells. These processes eventually contribute to the resolution of the damage and regeneration.122 As a recently discovered molecular link between thromboregulation and vascular inflammation, arterial wire injury was accompanied by a luminal upregulation of CD73/5′-ecto-nucleotidase expression, which limits not only thrombus formation but also subsequent neointima formation, endothelial activation, and mononuclear cell infiltration.191

Atherosclerosis is known to be influenced by a variety of chemokines and chemokine receptors. Many of the chemokines including the primarily described proatherogenic chemokine MCP-1 are found in platelets (Table 2). At present, we have collected only indirect hints of the role platelets play in this process. Bone marrow reconstitution in chimeras can provide certain clues; however, this alone is not sufficient to delineate the role of certain chemokines and mediators, which can also be synthesized by other hematopoietic cells. Clearly, platelet-specific conditional knockout models will be most instructive and essential to discriminate between the role platelets and other blood cells play in these complex processes.133 Indeed, PF4-Cre transgenic mice allowing the generation of lineage-restricted gene knockouts to study megakaryocyte and platelet function in vivo have recently been described.192

For some platelet chemokines, a predictive value for the risk of atherosclerosis has been revealed. The G403A RANTES polymorphism, which results in increased RANTES transcription, has been associated with increased risk for coronary atherosclerosis.193 Moreover, a correlation between the deposition of PF4 in carotid atherosclerotic plaques with lesion severity and symptomatic disease suggested that persistent platelet activation may contribute to the evolution of atherosclerosis.194 Given that platelet chemokines are most effective when immobilized on activated endothelium, this highlights that an analysis of chemokine levels in plasma alone may not be suitable to reflect their function and to serve as biomarkers in atherosclerosis. The deposition of chemokines by transiently interacting platelets may also explain why inhibition of aggregation by antiplatelet drugs, which does not interfere with this process, is not useful in preventing atherogenesis.

**Conclusions**

A variety of common molecular themes have recently accumulated to provide an intriguing link for the involvement of platelets between thrombosis, inflammatory reactions, and immune responses. Through their display of multiple adhesion molecules and receptors as outlined above, platelets are ideally suited to react with sites with vascular injury, where they bind to subendothelial molecules or activated endothelium as a prerequisite for clotting and wound healing. However, the same adhesion molecules also enable platelets to support leukocyte arrest but also to facilitate recruitment of leukocytes into inflamed tissue, where they exert their specific tasks in host defense and immunosurveillance, or to sites of vascular inflammation, namely atherosclerotic lesions. It has been well documented that platelets not only bind to different subsets of leukocytes, enhancing their contact with endothelium, but can also secrete mediators, triggering leukocyte arrest and inducing transendothelial migration. However, it remains to be elucidated whether platelets react uniformly to activating stimuli or whether, dependent on the stimulus, show different mobilization and modulation of their surface receptors and release of mediators, resulting in the recruitment of specific leukocyte subtypes. Recent findings extend the role of platelets in immune-driven settings, as they appear to serve as an interface between innate and acquired host defense, recognizing microbial pathogens...
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