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REVIEW

Platelet-neutrophil-interactions: Linking hemostasis and inflammation

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Summary Platelets are essential for primary hemostasis, but they also play an important pro-inflammatory role. Platelets normally circulate in a quiescent state. Upon activation, platelets can secrete and present various molecules, change their shape as well as the expression pattern of adhesion molecules. These changes are associated with the adhesion of platelets to leukocytes and the vessel wall. The interaction of platelets with neutrophils promotes the recruitment of neutrophils into inflammatory tissue and thus participates in host defense. This interaction of neutrophils with platelets is mainly mediated through P-selectin and β_2 and β_3 integrins (CD11b/CD18, CD41/CD61). Platelets can also interact with endothelial cells and monocytes. Adherent platelets promote the 'secondary capture' of neutrophils and other leukocytes. In addition, platelets secrete neutrophil and endothelial activators inducing production of inflammatory cytokines. Thus, platelets are important amplifiers of acute inflammation.

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Platelets

Platelets are un-nucleated fragments of bone marrow megakaryocytes. They contain few viable mitochondria, glycogen, at least three types of morphologically different granules (α -granules, dense core granules, lysosomes), and a complex membranous system. α -granules contain adhesion molecules important for platelet-platelet interac-

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tions and platelet interactions with other blood cells, mitogenic factors, plasma proteins, and factors relevant for coagulation and fibrinolysis (Table 1). Dense granules store small non-protein molecules such as ADP, ATP, serotonin, calcium and pyrophosphate, which play central roles in amplification of platelet aggregation and modulation of vascular endothelium and leukocyte function. Lysosomes contain glycosidases, proteases, and cationic proteins with bactericidal activity.¹ Secretion from lysosomal granules requires strong stimuli. Released hydrolytic enzymes digest material in platelet aggregates through hydrolytic degradation.¹

Platelets are involved in hemostasis, wound healing, and inflammation. Under physiological conditions, platelets circulate in a quiescent state, protected from untimely activation by inhibitory mediators released from intact endothelial cells, including nitric oxide (NO) and prostaglandin I₂ (PGI₂, prostacyclin). In addition, ectoADPase (CD39) removes extracellular ADP by converting it to adenosine. Endothelial dysfunction and changes in release of antiplatelet factors may lead to increased platelet activation followed by their inter-

action with neutrophils and monocytes, and increased platelet adhesion and aggregation.^{2,3} In one report, platelet adhesion to CD3+ T cells was observed.⁴ Both the recruitment and adhesion of platelets require specific adhesion molecules, chemokines, and their respective receptors (Tables 2 and 3). This review focuses on the molecules and platelet properties that link hemostasis and inflammation.

Platelet adhesion molecules

Integrins

Integrins are a large family of receptors which are constitutively expressed on the surface of almost all cells. They consist of transmembrane $\alpha\beta$ heterodimers and can bind extracellular matrix proteins as well as immunoglobulin-like adhesion molecules. Many cell-cell and cell-extracellular matrix interaction are regulated by integrins, which modulate important events in different biological processes, e.g. hemostasis, thrombosis, immunology,

Table 1 Contents of the three different granule subpopulations (α -granules, dense granules, and lysosomes) of platelets.¹

Dense granules	<p><i>Nucleotides</i></p> <p>Adenine: ATP, ADP Guanine: GTP, GDP</p> <p><i>Amines</i></p> <p>Serotonin Histamine</p> <p><i>Bivalent cations</i></p>
α -granules	<p><i>Adhesion molecules</i></p> <p>P-selectin (CD62P) Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) Glycoprotein IIb/IIIa (GPIIb/IIIa, $\alpha_{IIb}\beta_3$ integrin, CD41/CD61) von Willebrand factor (vWF) Thrombospondin-1 (TSP1) Vitronectin, Fibronectin</p> <p><i>Mitogenic factors</i></p> <p>Platelet-derived growth factor (PDGF) Vascular endothelial growth factor (VEGF) Transforming growth factor-β (TGF-β)</p> <p><i>Coagulation factors</i></p> <p>Fibrinogen, Plasminogen, Protein S, Kininogens Factors V, VII, XI, XIII</p> <p><i>Protease inhibitors</i></p> <p>C1 inhibitor Plasminogen activator inhibitor-1 (PAI-1) Tissue factor pathway inhibitor (TFPI)</p>
Lysosomes	<p><i>Glycosidases</i></p> <p><i>Proteases</i></p> <p><i>Cationic proteins</i></p>

Table 2 Cell-cell interactions require platelet surface molecules.

Surface molecules	Ligand
P-selectin	Binds PSGL-1 on neutrophils, monocytes, microparticles, and Th1 cells
ICAM-2	Binds LFA-1 on neutrophils and monocytes
vWF	Binds GPIb α
CD16 (mouse)/CD32 (human)	Obligatory coreceptor for GP VI
GPIb α	Binds vWF (mainly under high shear), P-selectin, and Mac-1
GPIIb/IIIa ($\alpha_{IIb}\beta_3$)	Binds FG, fibronectin, vitronectin, vWF, and thrombospondin
GP VI	Main platelet receptor for collagen
CD40L	Binds CD40 on monocytes and endothelial cells

Platelets possess different types of surface molecules which interact with corresponding molecules on platelets and other cells. ICAM, intercellular adhesion molecule; vWF, von Willebrand factor; FG, fibrinogen; LFA-1, lymphocyte function-associated antigen-1; PSGL-1, P-selectin glycoprotein ligand-1.

Table 3 Chemokines and chemokine-receptors of platelets.

Ligand	Receptor
CXCL1 (GRO- α)	CXCR2 (PMN)
CXCL4 (PF4)	
CXCL5 (ENA-78)	CXCR2 (PMN)
CXCL7 (NAP-2)	CXCR2 (PMN)
CXCL8 (IL-8)	CXCR1 (platelet and PMN) CXCR2 (PMN)
CCL3 (MIP-1 α)	CCR1, 5 (platelet)
CCL5 (RANTES)	CCR1, 3, 5 (platelet)
CCL7 (MCP-3)	CCR1, 2, 3 (platelet)

GRO- α , growth-related oncogene- α ; PF4, platelet factor 4; ENA-78, epithelial neutrophil-activating protein 78; NAP-2, neutrophil-activating protein-2; IL-8, interleukin-8; RANTES, regulated on activation, normal T cells expressed and secreted; MIP-1 α , macrophage inflammatory protein-1 α ; MCP-3, monocyte chemotactic protein-3; PMN, polymorphonuclear leukocytes.

inflammation, cell adhesion, growth, differentiation and spreading, angiogenesis and others.⁵

Most integrins require activation for ligand binding. Platelets express $\alpha_{IIb}\beta_3$ (GPIIb/IIIa), $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_2\beta_1$, and $\alpha_v\beta_3$ integrins.⁶ The activation and presentation of the ligand binding site of GPIIb/IIIa is initiated by placing the head domain of the intracellular talin molecule between the alpha- and beta-chains.⁷ This causes a change of conformation in the extracellular domains, followed by ligand binding, which causes further signaling that matures the bond.^{6,8,9} Integrins can amplify their binding capacity by forming clusters and patches on the cell surface.¹⁰

GPIb/IX/V

The major physiological role of the GPIb/IX/V glycoprotein complex is to mediate the initial adhe-

sion of circulating platelets to the exposed subendothelium or to intact proinflammatory endothelium under high shear stress. The complex is constitutively expressed on the platelet surface and consists of four distinct gene products: GPIb α , GPIb β , GPIX, and GPV. The most important ligand of the GIIb/IX/V complex is von Willebrand factor (vWF). The interaction occurs between the A1 domain of vWF and the N-terminal globular domain of GPIb α , which contains a series of leucine-rich repeats and an anionic peptide sequence with tyrosine sulfate residues.¹¹ Optimal binding to vWF requires tyrosine-sulfatation of GPIb α .¹² Several studies have demonstrated that vWF binds to more than one region of GPIb α , and the binding site depends on the type of activation.^{13,14} Under pathological stress conditions such as found in stenosed arteries, the binding of GPIb/IX/V complex to plasma vWF can initiate $\alpha_{IIb}\beta_3$ integrin activation via "outside-in" signaling¹⁵⁻¹⁸ associated with platelet shape change, secretion, aggregation, spreading, and contraction.¹⁹⁻²¹ Platelet GPIb α is also a ligand for endothelial P-selectin.²² Despite the lower affinity between these ligands, the very high density of GPIb α on platelet membranes allows rapid, shear-dependent platelet translocation (rolling) on surface bound P-selectin. GPIb α promotes platelet-platelet and platelet-endothelium interactions.²³ Macrophage antigen-1 (Mac-1; $\alpha_M\beta_2$ integrin, CD11b/CD18) can also bind directly to GPIb α .²⁴ Interaction between these two molecules involves the GPIb α leucine rich repeat and COOH-terminal flanking region and the α_M -subunit of Mac-1 (I domain), which is related to the A1 domain of vWF.²³

GPVI

GPVI is the main platelet collagen receptor. This 60-65 kDa molecule consists of two extracellular

immunoglobulin-like domain, a mucin stalk, a transmembrane domain, and a short cytoplasmatic chain.²⁵ The transmembrane domain possesses an arginine group that links GPVI to FcR γ -chain through a salt bridge. FcR γ -chain is composed of a disulphide-linked homodimer and two tyrosines arranged in a conserved sequence (immunoreceptor tyrosine-based activation motif (ITAM) domain). A proline-rich motif of the GPVI cytoplasmatic chain interacts with Src family tyrosine kinases, Fyn and Lyn. Collagen-mediated activation of the GPVI/FcR γ -chain complex through the cross-linking of two GPVI complexes leads to phosphorylation of the ITAM domain, which initiates intracellular signaling via the tyrosine kinase Syk activation followed by platelet adhesion and aggregation.²⁵

In addition to GPIb/IX/V and GPIIb/IIIa, GPVI is an important receptor in thrombus growth under high shear stress. The absence of GPVI on the surface of platelets is associated with impaired adhesion and thrombus formation,²⁶ and a mild bleeding predisposition.²⁷ This receptor is not directly involved in leukocyte-platelet interactions, but plays a crucial role for platelet recruitment, activation and their subsequent interaction with neutrophils through 'secondary capture'. 'Secondary capture' is the interaction of a freely flowing leukocyte with a rolling leukocyte or a platelet, which leads to subsequent attachment to the endothelium, and initiates rolling interactions.

GPIIb/IIIa ($\alpha_{IIb}\beta_3$ integrin)

The Glycoprotein IIb/IIIa integrin (CD41/CD61, $\alpha_{IIb}\beta_3$ integrin) is the most abundant platelet adhesion receptor.²⁸ The GPIIb/IIIa receptor is an important molecule for the aggregation of platelets and platelet-neutrophil-interaction. Under resting conditions, GPIIb/IIIa can bind immobilized fibrin(o-gen)²⁹ but not soluble ligands like fibronectin, fibrinogen, vitronectin, vWF, or thrombospondin (TSP)-1. The activation of GPIIb/IIIa by GPIb ligation and/or by G-protein-coupled receptors leads to a rapid conformational change of GPIIb/IIIa on the platelet membrane and the ability to bind soluble ligands.⁶ The inside-out activation of the GPIIb/IIIa-subunit involves changes in the conformation of both extracellular ligand-binding regions and the cytoplasmatic chains.¹⁰ Upon activation, platelet GPIIb/IIIa binds soluble extracellular adhesion molecules, such as vWF, fibrinogen, fibronectin, and thrombospondin. Furthermore, GPIIb/IIIa is responsible for the formation of fibrin bridges among platelets and is involved in platelet cohe-

sion and thrombus growth.³⁰ Following ligand binding, 'outside-in' signals influence platelet function (spreading and contraction) and the expression of adhesion molecules. GPIIa/IIIb is absent in Glanzmann thrombasthenia, which is associated with a severe bleeding due to defective platelet aggregation and clot retraction.^{31,32}

Von Willebrand factor (vWF)

Von Willebrand factor and P-selectin are stored in α -granules of platelets and in Weibel-Palade bodies of endothelial cells. Both are rapidly secreted upon activation.^{33,34} vWF is an adhesive glycoprotein present in plasma and in the subendothelial matrix in different conformations and activity states.³⁵ Endothelial cells are the major source of plasma vWF. vWF secreted from endothelial cells is rich in ultra large (UL) multimers and is normally cleaved by a disintegrin and metalloprotease with thrombospondin motif (ADAMTS) 13 into smaller and less active forms.^{36,37} The disruption of the balance between vWF release and cleavage by ADAMTS 13 can lead to an attachment of UL multimers to the cell surface. The presentation of UL vWF multimers induces platelet adhesion to the GPIb/IX/V complex and aggregation.³⁸

Selectins

Selectins are expressed on a wide range of vascular cells, including leukocytes, endothelial cells, and platelets. They are type I membrane proteins and contain a N-terminal C-type lectin domain, followed by an epidermal growth factor (EGF)-like motif, series of short consensus repeats, a transmembrane domain, and a cytoplasmatic chain. Selectins interact with cell-surface glycoconjugates and mediate tethering, rolling and adhesion of several types of cells.^{39,40} L-selectin is expressed on leukocytes, P-selectin is present on platelets and activated endothelial cells and E-selectin is present on activated endothelial cells. P-selectin plays an important role in neutrophil-platelet, platelet-platelet, and monocyte-platelet interactions (Table 2). Platelet P-selectin binds to P-selectin glycoprotein ligand-1 (PSGL-1)^{41,42} on neutrophils, monocytes, and a subset of Th1 cells,⁴³ thus promoting the initial binding of the cells. Firm platelet-neutrophil adhesion is mediated by integrin $\alpha_M\beta_2$ (CD11b/CD18 or Mac-1)^{44,45} binding to platelet GPIb²⁴ or to fibrinogen, which is bound to platelet GPIIb/IIIa or $\alpha_V\beta_3$ integrin.⁴⁶ A further mechanism of firm platelet-neutrophil adhesion is the interaction of platelet intracellular adhesion molecule

(ICAM)-2 with LFA-1 (CD11a/CD18).⁴⁷ The interaction between GPIIb α and platelet P-selectin also promotes the linkage among platelets.²³ Blocking of the initial (P-selectin-dependent) step using antibodies abolishes firm platelet adhesion to leukocytes in most experimental systems.^{48,49} It remains unclear whether PSGL-1 on platelets is an additional receptor for endothelial P-selectin.^{50,51}

G-protein-coupled receptors in platelets

Chemokine receptors

Chemokine receptors are members of the G-protein-coupled receptor family.^{52–55} Platelets express the chemokine receptors CCR1, CCR3, CCR4, CXCR1, and CXCR4 (Table 3) that bind proinflammatory and homeostatic chemokines. One important ligand for CCR4, which is present and functional on platelets,^{56,57} is CCL17 (Thymus and Activation Regulated Chemokine, TARC). This chemokine alone is not a potent platelet agonist, but it can enhance platelet stimulation in the presence of other agonists in an autocrine manner. CCR1, CCR3 and CXCR1 mRNAs were found in platelets, and CCR1 was also shown at the protein level.⁵⁷ Although the expression levels of CCR3 and CXCR1 are very low and could not be detected with antibodies, the functional relevance of both receptors was verified.^{56,57} Activation through these chemokine receptors enhances rather than initiates inflammatory processes, platelet aggregation, hemostasis, and thrombus formation.

Protease-activated receptors (PAR)

Four PAR receptors have so far been identified. Three are thrombin receptors (PAR-1, PAR-3, and PAR-4). Their structure is similar to that of other GPCRs. PAR-1, PAR-3, PAR-4 are expressed on platelets, whereas PAR-2 is expressed by a number of other cells, including endothelial cells, but not platelets. Thrombin binds to PAR 1, 3 and 4 and cleaves their amino terminal exodomain to unmask a new N-terminal end. This new amino terminus serves as a tethered ligand capable of receptor activation.⁵⁸ PAR-1, activated by thrombin, plays an important role in activation of human platelets. Blocking PAR-1 by antibodies inhibits human platelet activation by low, but not high, concentrations of thrombin.⁵⁹ In contrast to the importance of PAR-1 in human platelets, PAR-1 is not present on mouse platelets.⁶⁰ PAR-3 mediates the activation of mouse platelets upon stimulation with throm-

bin.⁶⁰ PAR-4 appears to function as a low-affinity thrombin receptor in both human and mouse platelets.⁶¹ In contrast to thrombin, other proteases including trypsin and tryptase activate PAR-2.⁶²

Thromboxane receptors

Thromboxane A₂ (TXA₂) is an important physiological activator of platelets and is produced by activated platelets through sequential enzymatic processing of arachidonic acid by phospholipase A₂, cyclooxygenase-1 and thromboxane synthase.⁶³ TXA₂ binds to the G-protein-coupled thromboxane A₂ receptor (TP), which induces platelet aggregation and vascular as well as respiratory smooth muscle contraction. There are two different types of TP known to date: TP α and TP β . Only TP α was detected in platelets.⁶⁴ TP couples to G α_q , G α_{12} and G α_{13} but not to G α_i .⁶⁵ The G α_q -subunit of the G-protein-coupled receptor activates phospholipase C- β (PLC- β), resulting in the production of diacylglycerol and inositol trisphosphate (IP₃). The elevation of cytosolic free Ca²⁺ by IP₃ and activation of protein kinase C by diacylglycerol lead to granule secretion and platelet shape change.⁶⁶ Additionally, shape change can directly be induced by two G $\alpha_{12/13}$ -subunit dependent pathways.^{65,67} This mechanism is based on TXA₂-induced secretion of ADP.^{68–70} After stimulation of TP by TXA₂, TP is rapidly desensitized and downregulated.

Adenosine diphosphate receptors

P2Y receptors are G-protein coupled receptors interacting with purine and pyrimidine nucleotides. The Gq-coupled P2Y1 receptor leads to activation of phospholipase C- β (PLC- β) upon stimulation by adenosine diphosphate (ADP). Stimulation of PLC- β induces mobilization of calcium with subsequent change of the platelet shape and transient aggregation.⁷¹ Inhibition or deletion of this receptor is associated with abnormal platelet aggregation and absence of shape changes.^{71,72} P2Y1 receptors also play a role in the initiation of platelet activation. The activation of the Gi-coupled receptor P2Y12 by ADP leads to an inhibition of adenylyl cyclase and activation of the γ isoform of phosphatidylinositol 3,4,5-trisphosphate (PI3K γ).⁶³ P2Y12 is involved in sustained, irreversible platelet aggregation.⁷³ P2Y12 is a specific target for antithrombotic drugs, e.g. ticlopidine and clopidogrel, which are clinically effective for prevention and treatment of vascular diseases.^{74–76}

In contrast to the P2Y receptors, P2X receptors are not G-protein coupled receptors but ligand-

gated ion channels containing two transmembrane domains, intracellular amino- and carboxyl termini, and a large extracellular loop with 10 conserved cysteine residues.⁷⁷ P2X1 receptors are present in human platelets. ATP, but not ADP, activates the P2X1 receptor and causes a rapid and selective change of membrane permeability for cations upon ligand binding.^{63,78}

Functional consequences of platelet activation

Shape change

Upon activation by thrombin, ADP or TXA₂, platelets undergo shape change, and secrete contents of α - and dense granules.⁷⁹ Rearrangement of cytoskeletal proteins, including the disassembly of a microtubule ring, occurs as one of the very first steps and results in a shape change from a disc-shaped cell into an intermediate spherical shape cell. This is followed by actin polymerization and extension of filopodia.^{80,81} Agonist-dependent phosphorylation of platelet myosin induces its polymerization and association with actin filaments.⁶⁵

Several independent ways of activation with a common final pathway can lead to platelet shape change. The common final pathway regulates the phosphorylation of myosin light chain (MLC) by MLC-kinase (MLCK) and myosin phosphatase. Activation of G α_q leads to a stimulation of PLC- β , elevation of diacylglycerol and IP₃. These alterations are accompanied by an increase of intracellular Ca²⁺ and activation of protein kinase C with subsequent regulation of MLCK-activity. Phosphorylation of MLC leads to actin-myosin interactions, resulting in actin-stimulated ATPase activity of smooth muscle and non-muscle myosin.⁸² Another pathway is the G $\alpha_{12/13}$ -Rho-Rho kinase pathway which can regulate the myosin-phosphatase-activity.⁶⁷ Tyrosine kinases are also involved in receptor-induced platelet shape change.^{67,83,84}

Secretion

Activated platelets secrete a number of potent inflammatory and mitogenic substances into the local microenvironment. These mediators modulate functions of other platelets, leukocytes, and endothelial cells. Platelets secrete chemokines (CCL3, 5, 7, 17, CXCL1, 4, 5, 7, and 8), cytokines (e.g., IL-1 β , CD40 ligand, β -thromboglobulin), growth factors (e.g., PDGF, TGF- β , EGF, VEGF, bFGF), and coagulation factors (e.g., factor V, factor XI,

PAI-1, plasminogen, protein S). These factors participate in cell survival, proliferation, coagulation and fibrinolysis, chemotaxis, and cell adhesion (Table 1).

Platelet chemokines (Table 3) play a key role in the activation of different cell types and can induce adhesion by activating integrins.^{85,86} Inflammatory platelet chemokines are found in both the CC- and the CXC-subfamily. CXC-chemokines can be further classified according to the presence of the tripeptide motif glutamic acid-leucine-arginine (ELR) in the NH₂-terminal region. All ELR⁺ CXC chemokines are proinflammatory.⁸⁷

Most platelet chemokines are stored in α -granules^{1,88} and can be released upon platelet activation. CXCL4 (platelet factor 4) is a chemokine that is constitutively and abundantly expressed in platelets. Endothelial CXCR3b, a splice variant of CXCR3, is a specific receptor for CXCL4 and may account for an angiostatic effect induced by activated platelets.⁸⁹ Chondroitin sulphate also binds CXCL4.⁹⁰ CXCL4 can activate neutrophils in the presence of appropriate co-stimuli such as tumor necrosis factor alpha (TNF- α). This combination of stimuli leads to exocytosis of the content of secondary granules of leukocytes (such as lactoferrin), but not primary granules or lysosomes.^{90–92} CXCL8 may be also an important chemokine for neutrophil recruitment and acts through CXCR1 and CXCR2 on (human) neutrophils.⁹³ CXCL7 activates neutrophils and thereby promotes chemotaxis, adhesion to endothelial cells, and degranulation of primary and secondary granules by binding its receptor CXCR2.^{88,91,94–97}

The CC-chemokines released by platelets do not have dramatic effects on neutrophils, but enhance paracrine activation of other platelets. Activated neutrophils up-regulate messenger RNA and protein levels of CCR-1 and become responsive to several CC-chemokines, such as CCL3, CCL5, and CCL7, which induce migration and Ca²⁺-mobilization.⁹⁸

The platelet cytokine CD40 ligand (CD40L), a transmembrane protein, was originally described on stimulated CD4⁺ T cells and also found on stimulated mast cells as well as basophils.⁹⁹ Preformed CD40L is stored in platelets and rapidly translocated to the cell surface upon activation. CD40L, surface-expressed or secreted by platelets, can bind to endothelial CD40 and induces chemokine secretion and upregulation of adhesion molecules.¹⁰⁰ This process leads to recruitment to and extravasation of leukocytes at the site of injury and thereby immediately links hemostasis to the inflammatory system.

Activated platelets secrete IL-1 β , a major activator of endothelial cells.^{101,102} Interaction of acti-

vated platelets with endothelial cells induces an IL-1 β dependent secretion of IL-6, CXCL8, CCL2 (monocyte chemoattractant protein-1) from endothelial cells.^{102,103} Beside the induction and release of these inflammatory mediators, IL-1 β induces an increased expression of adhesion molecules, such as E-selectin, VCAM-1, ICAM-1, $\alpha_v\beta_3$ integrin and others.¹⁰³

Two other important platelet agonists released upon activation are ADP and serotonin. ADP is stored both in dense granules and in the cytoplasm, but only the ADP of dense granules is released after platelet activation. ADP acts through P2Y1-receptor and produces Ca²⁺-mobilisation, shape changes, and initial transient activation.¹⁰⁴ It also interacts with the P2Y12-receptor, which mediates potentiation of platelet secretion and irreversible aggregation. Serotonin is an agonist of the G α_q -coupled 5HT2A-receptor and amplifies the platelet response.¹⁰⁴

Functional consequences of platelet-neutrophil interaction

Polymorphonuclear leukocytes (PMN) play an important role in host defense and in the pathogenesis of various diseases.¹⁰⁵ The recruitment of PMN into inflammatory tissue follows a distinct recruitment pattern. During these different recruitment steps, PMN become activated and subsequently release mediators into the surrounding tissue. In many experimental animal models, blockade of PMN recruitment or PMN depletion leads to attenuation of organ damage.^{106,107} In addition to observations in animal models, clinical studies show a positive correlation between the number of PMNs and the risk of acute myocardial infarction¹⁰⁸ as well as recurrence.¹⁰⁹

In addition to classical neutrophil recruitment, platelets bound to activated endothelial cells can interact with leukocytes, and induce 'secondary capture' (Table 4, Fig. 1) which induces interac-

tions of neutrophils with platelets first, followed by neutrophil-endothelial interaction.¹¹⁰

Even under high shear stress as may be encountered in arterioles or stenotic arteries platelets can adhere to subendothelial vWF. The interaction of platelets with vWF is mediated by the GIIb/IX/V complex. These interactions induce the activation of GPIIb/IIIa¹⁵⁻¹⁸ with a subsequent binding of GPIIb/IIIa to immobilized vWF, fibrinogen, and other ligands. The binding of integrin $\alpha_5\beta_1$ to fibronectin can also mediate stable adhesion.¹¹¹ Under low shear stress, adhesion of platelets can be mediated by integrins alone.¹¹²

Neutrophil rolling on platelets is mostly mediated by platelet P-selectin binding to P-selectin glycoprotein ligand (PSGL)-1 on leukocytes. Blocking one of these molecules with a mAb completely inhibits PMN rolling on platelets. Firm adhesion of leukocytes to platelets is achieved by CD11b/CD18 and CD11a/CD18. Further mechanisms involved in firm adhesion include the simultaneous binding of fibrinogen to GPIIb/IIIa on platelets and CD11b/CD18 on leukocytes⁴⁶ and the binding of GIIb to CD11b/CD18 (96). However, GPIIb/IIIa antagonists do not prevent the formation of platelet-neutrophil-aggregates in patients.¹¹³

Upon adhesion of PMN to platelets, activation of PMN is induced through PSGL-1^{114,115} and chemokine and lipid mediators presented by platelets.^{46,116} Platelet depletion reduces neutrophil rolling and adhesion in the brain microvasculature¹¹⁷ as well as leukocyte recruitment into the post-ischemic intestine.¹¹⁸ Not only stimulated platelets but also unstimulated platelets can roll on endothelial cells.³ This interaction is mediated by vWF transiently binding to endothelial cell P- and E-selectin.^{2,3,119} GIIb on platelets exhibits a high density but a low affinity to P-selectin and can mediate interactions with both P-selectin and vWF on stimulated endothelial cells. Platelet binding to endothelial cells can be blocked by mAb to either GIIb or vWF. Conversely, recruited leuko-

Table 4 Receptor-ligand pairs relevant for platelet interactions with endothelial cells and neutrophils.

Endothelial cells	↔	Platelet	↔	Neutrophil
CD40	↔	CD40L	↔	CD40
Fibrinogen, fibronectin	↔	GIIb/IIIa	↔	Mac-1
P-selectin, vWF	↔	GPIIb	↔	Mac-1, ICAM-1
		ICAM-2	↔	LFA-1
Unknown, possibly GPIIb	↔	P-selectin	↔	PSGL-1, L-selectin

Multicellular adhesive interactions occur among platelets, neutrophils, and endothelial cells. vWF, von Willebrand factor; ICAM, intracellular adhesion molecule; LFA-1, lymphocyte function-associated antigen-1; PSGL, P-selectin glycoprotein ligand; Mac-1, macrophage antigen-1.

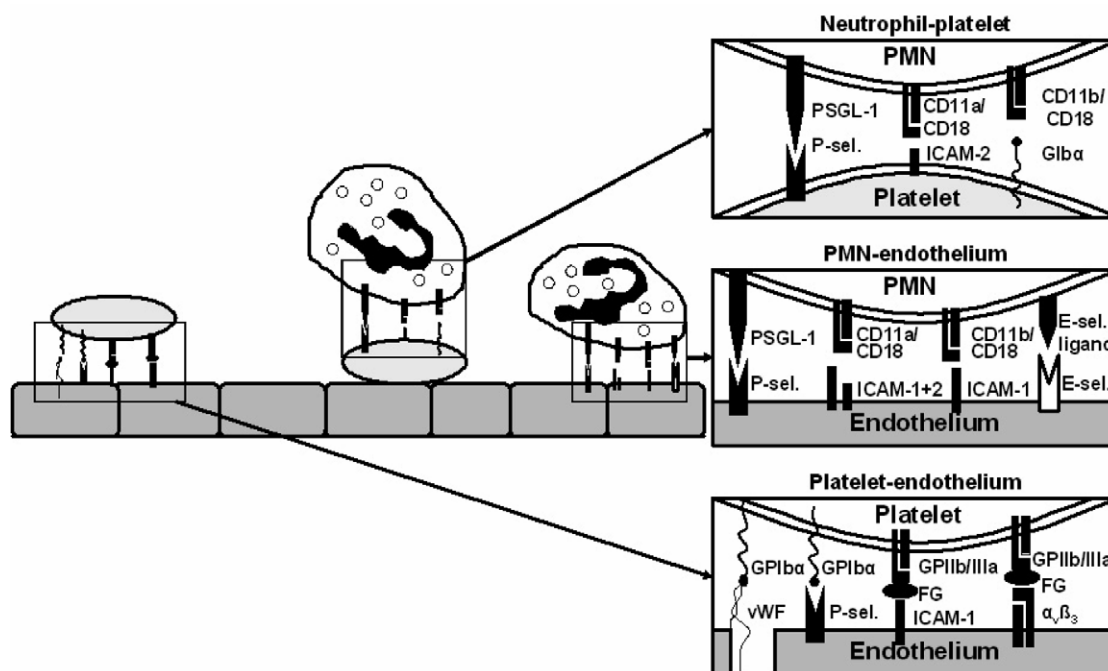


Figure 1 Platelet-independent- and platelet-dependent recruitment of PMN. PMN recruitment can occur either through the classical recruitment cascade or by adhering to platelets which are attached to the endothelial cells. Platelets adhere to inflamed endothelium via GPIIb/IIIa binding vWF and GPIIb/IIIa binding ICAM-1 and $\alpha_v\beta_3$ through fibrinogen bridges. vWF, von Willebrand factor; P-sel., P-selectin; E-sel., E-selectin; E-sel. lig, E-selectin ligand; ICAM, intracellular adhesion molecule; CD11a/CD18, lymphocyte function-associated antigen (LFA-1); CD11b/CD18, macrophage antigen-1 (Mac-1); PSGL, P-selectin glycoprotein ligand; $\alpha_v\beta_3$, integrin; FG, fibrinogen.

cytes can recruit circulating activated platelets through P-selectin-PSGL-1 interactions and contribute to further platelet activation through cathepsin G¹²⁰ and to fibrin deposition.¹²¹

In addition to interacting with neutrophils, platelets interact with other leukocyte subpopulations. Platelets present chemokines to and thereby activate monocytes.¹²² Activated platelets increase monocyte binding to inflamed endothelium, which is important in atherosclerosis.¹²³ The interaction between endothelial cells, platelets, and monocytes leads to increased monocyte recruitment and accelerates the development of atherosclerotic lesions.¹²³

Therapeutic inhibition of leukocyte-platelet aggregates can mitigate inflammatory processes and thereby the development of atherosclerosis and other inflammatory diseases. The *in vitro* inhibition of the P2Y₁₂-receptor by clopidogrel leads to inhibition of platelet P-selectin expression, platelet-PMN adhesion and production of ROS by PMN.¹²⁴ Furthermore, clopidogrel diminishes the ability of platelets to up-regulate the expression of tissue factor in monocytes.¹²⁴ These *in vitro* data correlate with clinical data. Long-term medication with clopidogrel showed a positive effect in the

prevention of adverse cardiac events after angioplasty and stenting.^{125,126} The combination of aspirin and clopidogrel has become standard treatment for one month after coronary stent implantation.¹²⁷ Adding clopidogrel to aspirin in the long-term management of patients with acute coronary syndromes without ST-segment elevation also demonstrated a higher efficacy.¹²⁸ Clopidogrel is also associated with a reduction in clinical parameters of infection.¹²⁹

Aspirin induces a complete and permanent inhibition of thromboxane A₂ production in platelets through the inactivation of cyclooxygenase-1 and -2 (COX-1, -2).¹³⁰ Several randomized clinical trials have shown that prevention of myocardial infarction and ischemic stroke by aspirin is largely due to inactivation of platelet COX-1.¹³¹ Aspirin reduces the risk of serious vascular events (nonfatal myocardial infarction, nonfatal stroke, or death from vascular causes) by approximately 25 percent.¹³¹ Aspirin is also used for prevention of atherothrombosis, chronic stable and unstable angina, severe carotid artery stenosis, acute cardiovascular events, and other indications.^{131,132} Due to its mechanism of action, aspirin can cause bleedings. However, the number in which a serious vascular

event is avoided outweighs the number with major bleeding episodes.

Another way to interrupt TXA₂ is to block the specific receptor with TP antagonists. These drugs have shown anti-thrombotic and cardioprotective activity in different animal models. Early TP antagonists produced disappointing results.¹³³ S-18886, a new TP antagonist, has completed clinical phase II with promising results.¹³⁴ Further TP antagonists are currently under development.

Regardless of the initiating stimulus, the final common pathway of platelet activation includes GPIIb/IIIa expression on the platelet surface. This glycoprotein is the target of several antiplatelet drugs.¹³⁵ Three types of GPIIb/IIIa inhibitors are available; a monoclonal antibody against the receptor, a nonpeptide, and a peptide.

The in vitro application of GPIIb/IIIa inhibitors prevents an increase of platelet P-selectin expression on the cell surface and reduces platelet-leukocyte interaction as well as release of PMN-elastase in a model of cardiopulmonary bypass.¹³⁶ For patients with acute coronary syndrome undergoing percutaneous coronary intervention, the combination of aspirin therapy and anti-GPIIb/IIIa is recommended to reduce the risk of procedure-related thrombotic complications.¹³³ In contrast to this recommendation, there is no consensus on the application of GPIIa/IIIb inhibitors for patients who are not scheduled for early revascularization.^{137,138} Oral long-term treatment with GPIIb/IIIa inhibitors is not more effective than aspirin or, when combined with aspirin, is not superior to aspirin plus placebo.^{139,140} Novel therapeutic approaches targeting GPVI and GPIb α are under experimental development. These treatments await full evaluation by clinical trials in a multicenter, double-blind, prospective setting.

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