Blood Reviews (2007) 21, 99–111

ELSEVIER

www.elsevierhealth.com/journals/blre

REVIEW

Platelet-neutrophil-interactions: Linking hemostasis and inflammation

Alexander Zarbock [a,e], Renata K. Polanowska-Grabowska [d], Klaus Ley [a,b,c,*]

[a] *Robert M. Berne Cardiovascular Research Center, University of Virginia; Charlottesville, Virginia, USA*
[b] *Department of Physiology and Biological Physics, University of Virginia; Charlottesville, Virginia, USA*
[c] *Department of Biomedical Engineering, University of Virginia; Charlottesville, Virginia, USA*
[d] *Department of Biochemistry & Molecular Genetics; University of Virginia; Charlottesville, Virginia, USA*
[e] *Department of Anesthesiology and Intensive Care Medicine, University of Münster, Münster, Germany*

KEYWORDS
Platelet-neutrophil-interactions;
Hemostasis;
Inflammation

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 $ß_2$ and $ß_3$ 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.

Platelets

Platelets are unnucleated 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

* Corresponding author. Present address: University of Virginia Health System, Robert M. Berne Cardiovascular Research Center P.O. Box 801394 Charlottesville, VA 22908-1394, USA: Tel.: +1 434 243-9966; fax: +1 434 924-2828.
E-mail addresses: az4n@virginia.edu (A. Zarbock), rp4t@mail.virginia.edu (R.K. Polanowska-Grabowska), klausley@virginia.edu (K. Ley).

0268-960X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.blre.2006.06.001

molecules important for platelet-platelet interactions 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 I_2 (PGI_2, 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 interaction 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 αß 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 pro-

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

Dense granules	*Nucleotides*
	Adenine: ATP, ADP
	Guanine: GTP, GDP
	Amines
	Serotonin
	Histamine
	Bivalent cations
α-granules	*Adhesion molecules*
	P-selectin (CD62P)
	Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31)
	Glycoprotein IIb/IIIa (GPIIb/IIIa, $\alpha_{IIb}ß_3$ integrin, CD41/CD61)
	von Willebrand factor (vWF)
	Thrombospondin-1 (TSP1)
	Vitronectin, Fibronectin
	Mitogenic factors
	Platelet-derived growth factor (PDGF)
	Vascular endothelial growth factor (VEGF)
	Transforming growth factor-ß (TGF-ß)
	Coagulation factors
	Fibrinogen, Plasminogen, Protein S, Kininogens
	Factors V, VII, XI, XIII
	Protease inhibitors
	C1 inhibitor
	Plasminogen activator inhibitor-1 (PAI-1)
	Tissue factor pathway inhibitor (TFPI)
Lysosomes	*Glycosidases*
	Proteases
	Cationic proteins

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 GPIbα
CD16 (mouse)/CD32 (human)	Obligatory coreceptor for GP VI
GPIbα	Binds vWF (mainly under high shear), P-selectin, and Mac-1
GPIIb/IIIa ($\alpha_{IIb}ß_3$)	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-1α)	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-1α, macrophage inflammatory protein-1α; MCP-3, monocyte chemotactic protein-3; PMN, polymorphonuclear leukocytes.

cesses, e.g. hemostasis, thrombosis, immunology, inflammation, cell adhesion, growth, differentiation and spreading, angiogenesis and others.[5]

Most integrins require activation for ligand binding. Platelets express $\alpha_{IIb}ß_3$ (GPIIb/IIIa), $\alpha_5ß_1$, $\alpha_6ß_1$, $\alpha_2ß_1$, and $\alpha_vß_3$ 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 adhesion 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: GPIbα, GPIbß, GPIX, and GPV. The most important ligand of the GIb/IX/V complex is von Willebrand factor (vWF). The interaction occurs between the A1 domain of vWF and the N-terminal globular domain of GPIbα, 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 GPIbα.[12] Several studies have demonstrated that vWF binds to more than one region of GPIbα, 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 $\alpha_{IIb}ß_3$ integrin activation via ''outside-in'' signaling[15—18] associated with platelet shape change, secretion, aggregation, spreading, and contraction.[19—21] Platelet GPIbα is also a ligand for endothelial P-selectin.[22] Despite the lower affinity between these ligands, the very high density of GPIbα on platelet membranes allows rapid, shear—dependent platelet translocation (rolling) on surface bound P-selectin. GPIbα promotes platelet-platelet and platelet-endothelium interactions.[23] Macrophage antigen-1 (Mac-1; $\alpha_Mß_2$ integrin, CD11b/CD18) can also bind directly to GPIbα.[24] Interaction between these two molecules involves the GPIbα 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 ($\alpha_{IIb}ß_3$ integrin)

The Glycoprotein IIb/IIIa integrin (CD41/CD61, $\alpha_{IIb}ß_3$ integrin) is the most abundant platelet adhesion receptor.[28] The GIIb/IIIa receptor is an important molecule for the aggregation of platelets and platelet-neutrophil-interaction. Under resting conditions, GPIIb/IIIa can bind immobilized fibrin(ogen)[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 cohesion 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 $\alpha_M ß_2$ (CD11b/CD18 or Mac-1)[44,45] binding to platelet GPIb[24] or to fibrinogen, which is bound to platelet GIIb/IIIa or $\alpha_V ß_3$ 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 GPIbα 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 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 thrombin.[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 A_2 (TXA_2) is an important physiological activator of platelets and is produced by activated platelets through sequential enzymatic processing of arachidonic acid by phospholipase A_2, cyclooxygenase-1 and thromboxane synthase.[63] TXA_2 binds to the G-protein-coupled thromboxane A_2 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: TPα and TPß. Only TPα was detected in platelets.[64] TP couples to $G\alpha_q$, $G\alpha_{12}$ and $G\alpha_{13}$ but not to $G\alpha_i$.[65] The $G\alpha_q$-subunit of the G-protein-coupled receptor activates phospholipase C-ß (PLC-ß), resulting in the production of diacylglycerol and inositol trisphosphate (IP_3). The elevation of cytosolic free Ca^{2+} by IP_3 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 TXA_2-induced secretion of ADP.[68–70] After stimulation of TP by TXA_2, 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 phopholipase C-ß (PLC-ß) upon stimulation by adenosine diphosphate (ADP). Stimulation of PLC-ß 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 (PI3Kγ).[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 TXA_2, 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 $G\alpha_q$ leads to a stimulation of PLC-ß, elevation of diacylglycerol and IP_3. These alterations are accompanied by an increase of intracellular Ca^{2+} 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-1β, 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 NH_2-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 Ca^{2+}-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-1ß, a major activator of endothelial cells.[101,102] Interaction of acti-

vated platelets with endothelial cells induces an IL-1ß 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-1ß induces an increased expression of adhesion molecules, such as E-selectin, VCAM-1, ICAM-1, $\alpha_v ß_3$ 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 Ca^{2+}-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 $G\alpha_q$-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 infarction[108] 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 interactions 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 GIb/IX/V complex. These interactions induce the activation of GPIIb/IIIa[15–18] with a subsequent binding of GPIIb/IIIa to immobilized vWF, fibrinogen, and other ligands. The binding of integrin $\alpha_5 ß_1$ 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 leukocytes[46] and the binding of GIbα 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 microvasculature[117] 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] GIbα 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 GIbα or vWF. Conversely, recruited

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	↔	GPIbα	↔	Mac-1, ICAM-1
		ICAM-2	↔	LFA-1
Unknown, possibly GPIbα	↔	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.

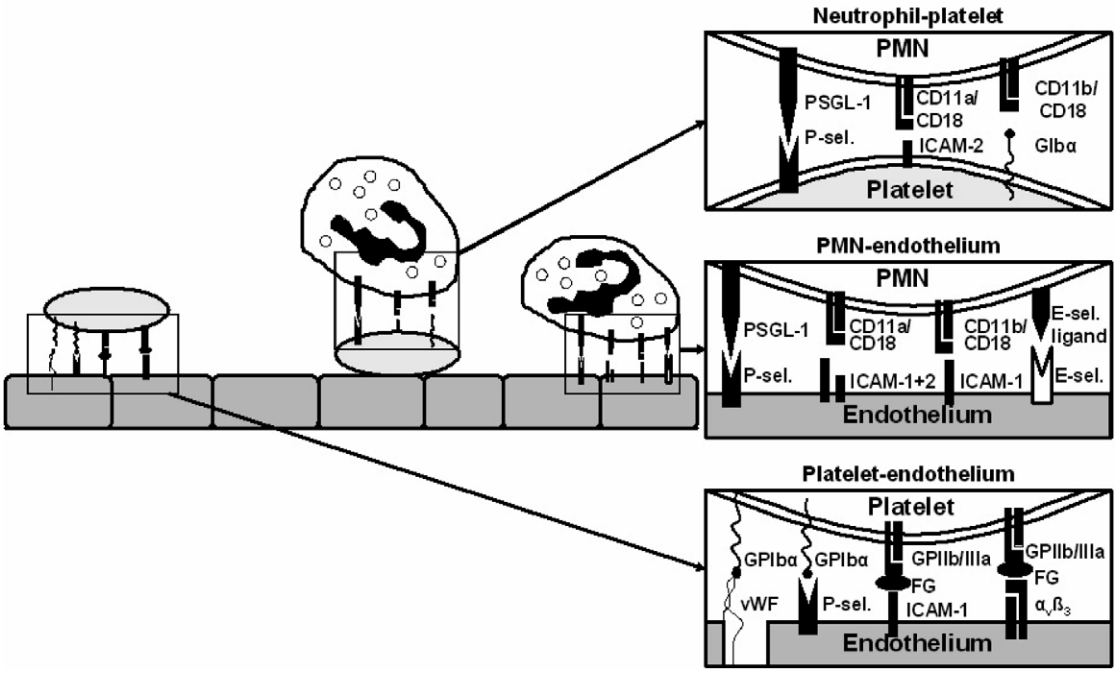

Figure 1 Platelet-independent- and platelet-dependent recruitment of PMN. PMN recruitment can occur either through the classical recruitment cascade or by adhering to platelets which are attached to the endothelial cells. Platelets adhere to inflamed endothelium via GPIbα binding vWF and GIIb/IIIa binding ICAM-1 and $\alpha_v ß_3$ 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; $\alpha_v ß_3$, integrin; FG, fibrinogen.

leukocytes can recruit circulating activated platelets through P-selectin-PSGL-1 interactions and contribute to further platelet activation through cathepsin G[120] 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 in 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] Clopidrogrel is also associated with a reduction in clinical parameters of infection.[129]

Aspirin induces a complete and permanent inhibition of thromboxane A_2 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 TXA_2 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 GIIb/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 GIIb/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 GPIbα are under experimental development. These treatments await full evaluation by clinical trials in a multicenter, double-blind, prospective setting.

Acknowledgement

A.Z. is supported by a grant of the Deutsche Forschungsgemeinschaft (DFG AZ 428/2—1). The original work from K.L.'s lab is supported by grants from the National Institutes of Health HL58108, 55798 and 73361.

References

1. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and functions. *Platelets*. 2001;**12**:261—73.
2. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci U S A*. 1995;**92**:7450—4.
3. Frenette PS, Moyna C, Hartwell DW, Lowe JB, Hynes RO, Wagner DD. Platelet-endothelial interactions in inflamed mesenteric venules. *Blood*. 1998;**91**:1318—24.
4. Diacovo TG, Puri KD, Warnock RA, Springer TA, von Andrian UH. Platelet-mediated lymphocyte delivery to high endothelial venules. *Science*. 1996;**273**:252—5.
5. Schwartz MA, Schaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. *Annu Rev Cell Dev Biol*. 1995;**11**:549—99.
6. Bennett JS. Structure and function of the platelet integrin alphaIIbbeta3. *J Clin Invest*. 2005;**115**:3363—9.
7. Tadokoro S, Shattil SJ, Eto K, et al. Talin binding to integrin beta tails: a final common step in integrin activation. *Science*. 2003;**302**:103—6.
8. Alon R, Grabovsky V, Feigelson S. Chemokine induction of integrin adhesiveness on rolling and arrested leukocytes local signaling events or global stepwise activation? *Microcirculation*. 2003;**10**:297—311.
9. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002;**110**:673—87.
10. Xiong JP, Stehle T, Goodman SL, Arnaout MA. New insights into the structural basis of integrin activation. *Blood*. 2003;**102**:1155—9.
11. Cruz MA, Diacovo TG, Emsley J, Liddington R, Handin RI. Mapping the glycoprotein Ib-binding site in the von willebrand factor A1 domain. *J Biol Chem*. 2000;**275**:19098—105.
12. Dong JF, Hyun W, Lopez JA. Aggregation of mammalian cells expressing the platelet glycoprotein (GP) Ib-IX complex and the requirement for tyrosine sulfation of GP Ib alpha. *Blood*. 1995;**86**:4175—83.
13. Ward CM, Andrews RK, Smith AI, Berndt MC. Mocarhagin, a novel cobra venom metalloproteinase, cleaves the platelet von Willebrand factor receptor glycoprotein Ibalpha. Identification of the sulfated tyrosine/anionic sequence Tyr-276-Glu-282 of glycoprotein Ibalpha as a binding site for von Willebrand factor and alpha-thrombin. *Biochemistry*. 1996;**35**:4929—38.
14. Marchese P, Murata M, Mazzucato M, et al. Identification of three tyrosine residues of glycoprotein Ib alpha with distinct roles in von Willebrand factor and alpha-thrombin binding. *J Biol Chem*. 1995;**270**:9571—8.
15. Yuan Y, Kulkarni S, Ulsemer P, et al. The von Willebrand factor-glycoprotein Ib/V/IX interaction induces actin polymerization and cytoskeletal reorganization in rolling platelets and glycoprotein Ib/V/IX-transfected cells. *J Biol Chem*. 1999;**274**:36241—51.
16. Gu M, Xi X, Englund GD, Berndt MC, Du X. Analysis of the roles of 14-3-3 in the platelet glycoprotein Ib-IX-mediated activation of integrin alpha(IIb)beta(3) using a reconstituted mammalian cell expression model. *J Cell Biol*. 1999;**147**:1085—96.
17. Yap CL, Hughan SC, Cranmer SL, et al. Synergistic adhesive interactions and signaling mechanisms operating between platelet glycoprotein Ib/IX and integrin alpha IIbbeta 3. Studies in human platelets ans transfected Chinese hamster ovary cells. *J Biol Chem*. 2000;**275**:41377—88.
18. Zaffran Y, Meyer SC, Negrescu E, Reddy KB, Fox JE. Signaling across the platelet adhesion receptor glycoprotein Ib-IX induces alpha IIbbeta 3 activation both in platelets and a transfected Chinese hamster ovary cell system. *J Biol Chem*. 2000;**275**:16779—87.
19. Kroll MH, Hellums JD, McIntire LV, Schafer AI, Moake JL. Platelets and shear stress. *Blood*. 1996;**88**:1525—41.

20. Ruggeri ZM. Mechanisms initiating platelet thrombus formation. *Thromb Haemost*. 1997;**78**:611—6.
21. Andrews RK, Berndt MC. Adhesion-dependent signalling and the initiation of haemostasis and thrombosis. *Histol Histopathol*. 1998;**13**:837—44.
22. Romo GM, Dong JF, Schade AJ, et al. The glycoprotein Ib-IX-V complex is a platelet counterreceptor for P-selectin. *J Exp Med*. 1999;**190**:803—14.
23. Andrews RK, Berndt MC. Platelet physiology and thrombosis. *Thromb Res*. 2004;**114**:447—53.
24. Simon DI, Chen Z, Xu H, et al. Platelet glycoprotein ibalpha is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J Exp Med*. 2000;**192**:193—204.
25. Nieswandt B, Watson SP. Platelet-collagen interaction: is GPVI the central receptor? *Blood*. 2003;**102**:449—61.
26. Massberg S, Gawaz M, Gruner S, et al. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. *J Exp Med*. 2003;**197**:41—9.
27. Sarratt KL, Chen H, Zutter MM, Santoro SA, Hammer DA, Kahn ML. GPVI and alpha2beta1 play independent critical roles during platelet adhesion and aggregate formation to collagen under flow. *Blood*. 2005;**106**:1268—77.
28. Leclerc JR. Platelet glycoprotein IIb/IIIa antagonists: lessons learned from clinical trials and future directions. *Crit Care Med*. 2002;**30**:S332—40.
29. Luscher EF, Weber S. The formation of the haemostatic plug—a special case of platelet aggregation. An experiment and a survey of the literature. *Thromb Haemost*. 1993;**70**:234—7.
30. Dubois C, Reigner SC, Steiner B, Riederer MA. Thrombin binding to GPIbalpha induces integrin alphaIIbbeta3 dependent platelet adhesion to fibrin in ex vivo flowing whole blood. *Thromb Haemost*. 2004;**91**:233—7.
31. Hodivala-Dilke KM, McHugh KP, Tsakiris DA, et al. Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. *J Clin Invest*. 1999;**103**:229—38.
32. Tronik-Le Roux D, Roullot V, Poujol C, Kortulewski T, Nurden P, Marguerie G. Thrombasthenic mice generated by replacement of the integrin alpha(IIb) gene: demonstration that transcriptional activation of this megakaryocytic locus precedes lineage commitment. *Blood*. 2000;**96**:1399—408.
33. Tsai HM, Nagel RL, Hatcher VB, Seaton AC, Sussman II. The high molecular weight form of endothelial cell von Willebrand factor is released by the regulated pathway. *Br J Haematol*. 1991;**79**:239—45.
34. Sadler JE. Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem*. 1998;**67**:395—424.
35. Ruggeri ZM. Von Willebrand factor, platelets and endothelial cell interactions. *J Thromb Haemost*. 2003;**1**:1335—42.
36. Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*. 2001;**413**:488—94.
37. Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem*. 2001;**276**:41059—63.
38. Dong JF, Moake JL, Nolasco L, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*. 2002;**100**:4033—9.
39. Ley K. The role of selectins in inflammation and disease. *Trends Mol Med*. 2003;**9**:263—8.
40. Kansas GS. Selectins and their ligands: current concepts and controversies. *Blood*. 1996;**88**:3259—87.
41. Yang J, Furie BC, Furie B. The biology of P-selectin glycoprotein ligand-1: its role as a selectin counterreceptor in leukocyte-endothelial and leukocyte-platelet interaction. *Thromb Haemost*. 1999;**81**:1—7.
42. Evangelista V, Manarini S, Sideri R, et al. Platelet/polymorphonuclear leukocyte interaction: P-selectin triggers protein-tyrosine phosphorylation-dependent CD11b/CD18 adhesion: role of PSGL-1 as a signaling molecule. *Blood*. 1999;**93**:876—85.
43. Ley K, Kansas GS. Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. *Nat Rev Immunol*. 2004;**4**:325—35.
44. Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. *Blood*. 1996;**88**:146—57.
45. Evangelista V, Manarini S, Rotondo S, et al. Platelet/polymorphonuclear leukocyte interaction in dynamic conditions: evidence of adhesion cascade and cross talk between P-selectin and the beta 2 integrin CD11b/CD18. *Blood*. 1996;**88**:4183—94.
46. Weber C, Springer TA. Neutrophil accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to alphaIIbbeta3 and stimulated by platelet-activating factor. *J Clin Invest*. 1997;**100**:2085—93.
47. Diacovo TG, deFougerolles AR, Bainton DF, Springer TA. A functional integrin ligand on the surface of platelets: intercellular adhesion molecule-2. *J Clin Invest*. 1994;**94**:1243—51.
48. da Costa Martins PA, van Gils JM, Mol A, Hordijk PL, Zwaginga JJ. Platelet binding to monocytes increases the adhesive properties of monocytes by up-regulating the expression and functionality of {beta}1 and {beta}2 integrins. *J Leukoc Biol*.
49. Pitchford SC, Momi S, Giannini S, et al. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood*. 2005;**105**:2074—81.
50. Frenette PS, Denis CV, Weiss L, et al. P-Selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. *J Exp Med*. 2000;**191**:1413—22.
51. Sperandio M, Smith ML, Forlow SB, et al. P-selectin glycoprotein ligand-1 mediates L-selectin-dependent leukocyte rolling in venules. *J Exp Med*. 2003;**197**:1355—63.
52. Horuk R. Molecular properties of the chemokine receptor family. *Trends Pharmacol Sci*. 1994;**15**:159—65.
53. Locati M, Murphy PM. Chemokines and chemokine receptors: biology and clinical relevance in inflammation and AIDS. *Annu Rev Med*. 1999;**50**:425—40.
54. Rodriguez-Frade JM, Mellado M, Martinez AC. Chemokine receptor dimerization: two are better than one. *Trends Immunol*. 2001;**22**:612—7.
55. Thelen M. Dancing to the tune of chemokines. *Nat Immunol*. 2001;**2**:129—34.
56. Gear AR, Suttitanamongkol S, Viisoreanu D, Polanowska-Grabowska RK, Raha S, Camerini D. Adenosine diphosphate strongly potentiates the ability of the chemokines MDC, TARC, and SDF-1 to stimulate platelet function. *Blood*. 2001;**97**:937—45.
57. Clemetson KJ, Clemetson JM, Proudfoot AE, Power CA, Baggiolini M, Wells TN. Functional expression of CCR1, CCR3, CCR4, and CXCR4 chemokine receptors on human platelets. *Blood*. 2000;**96**:4046—54.

58. Savage B, Cattaneo M, Ruggeri ZM. Mechanisms of platelet aggregation. *Curr Opin Hematol*. 2001;**8**:270—6.
59. Coughlin SR. Protease-activated receptors and platelet function. *Thromb Haemost*. 1999;**82**:353—6.
60. Connolly AJ, Ishihara H, Kahn ML, Farese Jr RV. Coughlin SR. Role of the thrombin receptor in development and evidence for a second receptor. *Nature*. 1996;**381**:516—9.
61. Kahn ML, Zheng YW, Huang W, et al. A dual thrombin receptor system for platelet activation. *Nature*. 1998;**394**:690—4.
62. Steinhoff M, Buddenkotte J, Shpacovitch V, et al. Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. *Endocr Rev*. 2005;**26**:1—43.
63. Murugappan S, Shankar H, Kunapuli SP. Platelet receptors for adenine nucleotides and thromboxane A2. *Semin Thromb Hemost*. 2004;**30**:411—8.
64. Habib A, FitzGerald GA, Maclouf J. Phosphorylation of the thromboxane receptor alpha, the predominant isoform expressed in human platelets. *J Biol Chem*. 1999;**274**:2645—51.
65. Offermanns S. The role of heterotrimeric G proteins in platelet activation. *Biol Chem*. 2000;**381**:389—96.
66. Hirata T, Ushikubi F, Kakizuka A, Okuma M, Narumiya S. Two thromboxane A2 receptor isoforms in human platelets. Opposite coupling to adenylyl cyclase with different sensitivity to Arg60 to Leu mutation. *J Clin Invest*. 1996;**97**:949—56.
67. Klages B, Brandt U, Simon MI, Schultz G, Offermanns S. Activation of G12/G13 results in shape change and Rho/Rho-kinase-mediated myosin light chain phosphorylation in mouse platelets. *J Cell Biol*. 1999;**144**:745—54.
68. Pulcinelli FM, Ashby B, Gazzaniga PP, Daniel JL. Protein kinase C activation is not a key step in ADP-mediated exposure of fibrinogen receptors on human platelets. *FEBS Lett*. 1995;**364**:87—90.
69. Pulcinelli FM, Ciampa MT, Favilla M, Pignatelli P, Riondino PP, Gazzaniga PP. Concomitant activation of Gi protein-coupled receptor and protein kinase C or phospholipase C is required for platelet aggregation. *FEBS Lett*. 1999;**460**:37—40.
70. Paul BZ, Jin J, Kunapuli SP. Molecular mechanism of thromboxane A(2)-induced platelet aggregation. Essential role for p2t(ac) and alpha(2a) receptors. *J Biol Chem*. 1999;**274**:29108—14.
71. Fabre JE, Nguyen M, Latour A, et al. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice. *Nat Med*. 1999;**5**:1199—202.
72. Geiger J, Honig-Liedl P, Schanzenbacher P, Walter U. Ligand specificity and ticlopidine effects distinguish three human platelet ADP receptors. *Eur J Pharmacol*. 1998;**351**:235—46.
73. Dorsam RT, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. *J Clin Invest*. 2004;**113**:340—5.
74. Foster CJ, Prosser DM, Agans JM, et al. Molecular identification and characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs. *J Clin Invest*. 2001;**107**:1591—8.
75. Hollopeter G, Jantzen HM, Vincent D, et al. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature*. 2001;**409**:202—7.
76. Zhang FL, Luo L, Gustafson E, et al. ADP is the cognate ligand for the orphan G protein-coupled receptor SP1999. *J Biol Chem*. 2001;**276**:8608—15.
77. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev*. 1998;**50**:413—92.
78. Valera S, Hussy N, Evans RJ, et al. A new class of ligand-gated ion channel defined by P2x receptor for extracellular ATP. *Nature*. 1994;**371**:516—9.
79. Holmsen H. Significance of testing platelet functions in vitro. *Eur J Clin Invest*. 1994;**24 Suppl 1**:3—8.
80. Gachet C. The platelet P2 receptors as molecular targets for old and new antiplatelet drugs. *Pharmacol Ther*. 2005;**108**:180—92.
81. Bearer EL. Cytoskeletal domains in the activated platelet. *Cell Motil Cytoskeleton*. 1995;**30**:50—66.
82. Somlyo AP, Somlyo AV. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *J Physiol*. 2000;**522**(Pt 2):177—85.
83. Gallet C, Rosa JP, Habib A, Lebret M, Levy-Toledano S, Maclouf J. Tyrosine phosphorylation of cortactin associated with Syk accompanies thromboxane analogue-induced platelet shape change. *J Biol Chem*. 1999;**274**:23610—6.
84. Negrescu EV, Siess W. Dissociation of the alphaIIbbeta3-integrin by EGTA stimulates the tyrosine kinase pp72(syk) without inducing platelet activation. *J Biol Chem*. 1996;**271**:26547—53.
85. Detmers PA, Lo SK, Olsen-Egbert E, Walz A, Baggiolini M, Cohn ZA. Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. *J Exp Med*. 1990;**171**:1155—62.
86. Carveth HJ, Bohnsack JF, McIntyre TM, Baggiolini M, Prescott SM, Zimmerman GA. Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cells and to subendothelial matrix proteins. *Biochem Biophys Res Commun*. 1989;**162**:387—93.
87. Olson TS, Ley K. Chemokines and chemokine receptors in leukocyte trafficking. *Am J Physiol Regul Integr Comp Physiol*. 2002;**283**:R7—R28.
88. Brandt E, Ludwig A, Petersen F, Flad HD. Platelet-derived CXC chemokines: old players in new games. *Immunol Rev*. 2000;**177**:204—16.
89. Lasagni L, Francalanci M, Annunziato F, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. *J Exp Med*. 2003;**197**:1537—49.
90. Petersen F, Bock L, Flad HD, Brandt E. Platelet factor 4-induced neutrophil-endothelial cell interaction: involvement of mechanisms and functional consequences different from those elicited by interleukin-8. *Blood*. 1999;**94**:4020—8.
91. Brandt E, Petersen F, Ludwig A, Ehlert JE, Bock L, Flad HD. The beta-thromboglobulins and platelet factor 4: blood platelet-derived CXC chemokines with divergent roles in early neutrophil regulation. *J Leukoc Biol*. 2000;**67**:471—8.
92. Petersen F, Ludwig A, Flad HD, Brandt E. TNF-alpha renders human neutrophils responsive to platelet factor 4. Comparison of PF-4 and IL-8 reveals different activity profiles of the two chemokines. *J Immunol*. 1996;**156**:1954—62.
93. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. *Adv Immunol*. 1994;**55**:97—179.
94. Ludwig A, Petersen F, Zahn S, et al. The CXC-chemokine neutrophil-activating peptide-2 induces two distinct optima of neutrophil chemotaxis by differential interaction with interleukin-8 receptors CXCR-1 and CXCR-2. *Blood*. 1997;**90**:4588—97.
95. Kasper B, Brandt E, Ernst M, Petersen F. Neutrophil adhesion to endothelial cells induced by platelet factor 4

(PF4; CXCL4) requires sequential activation of Ras, Syk, and JNK MAP kinases. *Blood*. 2005.
96. Piccardoni P, Evangelista V, Piccoli A, de Gaetano G, Walz C, Cerletti C. Thrombin-activated human platelets release two NAP-2 variants that stimulate polymorphonuclear leukocytes. *Thromb Haemost*. 1996;**76**:780–5.
97. Aziz KA, Cawley JC, Zuzel M. Platelets prime PMN via released PF4: mechanism of priming and synergy with GM-CSF. *Br J Haematol*. 1995;**91**:846–53.
98. Cheng SS, Lai JJ, Lukacs NW, Kunkel SL. Granulocyte-macrophage colony stimulating factor up-regulates CCR1 in human neutrophils. *J Immunol*. 2001;**166**:1178–84.
99. Vishnevetsky D, Kiyanista VA, Gandhi PJ. CD40 ligand: a novel target in the fight against cardiovascular disease. *Ann Pharmacother*. 2004;**38**:1500–8.
100. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*. 1998;**391**:591–4.
101. Hawrylowicz CM, Howells GL, Feldmann M. Platelet-derived interleukin 1 induces human endothelial adhesion molecule expression and cytokine production. *J Exp Med*. 1991;**174**:785–90.
102. Kaplanski G, Farnarier C, Kaplanski S, et al. Interleukin-1 induces interleukin-8 secretion from endothelial cells by a juxtacrine mechanism. *Blood*. 1994;**84**:4242–8.
103. Gawaz M, Brand K, Dickfeld T, et al. Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. *Implications for atherogenesis. Atherosclerosis*. 2000;**148**:75–85.
104. Gachet C. ADP receptors of platelets and their inhibition. *Thromb Haemost*. 2001;**86**:222–32.
105. Liu Y, Shaw SK, Ma S, Yang L, Luscinskas FW, Parkos CA. Regulation of leukocyte transmigration: cell surface interactions and signaling events. *J Immunol*. 2004;**172**:7–13.
106. Singbartl K, Green SA, Ley K. Blocking P-selectin protects from ischemia/reperfusion-induced acute renal failure. *Faseb J*. 2000;**14**:48–54.
107. Sisley AC, Desai T, Harig JM, Gewertz BL. Neutrophil depletion attenuates human intestinal reperfusion injury. *J Surg Res*. 1994;**57**:192–6.
108. Ernst E, Hammerschmidt DE, Bagge U, Matrai A, Dormandy JA. Leukocytes and the risk of ischemic diseases. *Jama*. 1987;**257**:2318–24.
109. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *Jama*. 1998;**279**:1477–82.
110. Mine S, Fujisaki T, Suematsu M, Tanaka Y. Activated platelets and endothelial cell interaction with neutrophils under flow conditions. *Intern Med*. 2001;**40**:1085–92.
111. Savage B, Almus-Jacobs F, Ruggeri ZM. Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. *Cell*. 1998;**94**:657–66.
112. Ruggeri ZM, Dent JA, Saldivar E. Contribution of distinct adhesive interactions to platelet aggregation in flowing blood. *Blood*. 1999;**94**:172–8.
113. Zhao L, Bath PM, May J, Losche W, Heptinstall S. P-selectin, tissue factor and CD40 ligand expression on platelet-leucocyte conjugates in the presence of a GPIIb/IIIa antagonist. *Platelets*. 2003;**14**:473–80.
114. Blanks JE, Moll T, Eytner R, Vestweber D. Stimulation of P-selectin glycoprotein ligand-1 on mouse neutrophils activates beta 2-integrin mediated cell attachment to ICAM-1. *Eur J Immunol*. 1998;**28**:433–43.
115. Hidari KI, Weyrich AS, Zimmerman GA, McEver RP. Engagement of P-selectin glycoprotein ligand-1 enhances tyrosine phosphorylation and activates mitogen-activated protein kinases in human neutrophils. *J Biol Chem*. 1997;**272**:28750–6.
116. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappa B translocation. *J Clin Invest*. 1995;**95**:2297–303.
117. Carvalho-Tavares J, Hickey MJ, Hutchison J, Michaud J, Sutcliffe IT, Kubes P. A role for platelets and endothelial selectins in tumor necrosis factor-alpha-induced leukocyte recruitment in the brain microvasculature. *Circ Res*. 2000;**87**:1141–8.
118. Salter JW, Krieglstein CF, Issekutz AC, Granger DN. Platelets modulate ischemia/reperfusion-induced leukocyte recruitment in the mesenteric circulation. *Am J Physiol Gastrointest Liver Physiol*. 2001;**281**:G1432–9.
119. Andre P, Denis CV, Ware J, et al. Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. *Blood*. 2000;**96**:3322–8.
120. de Gaetano G, Cerletti C, Evangelista V. Recent advances in platelet-polymorphonuclear leukocyte interaction. *Haemostasis*. 1999;**29**:41–9.
121. Palabrica T, Lobb R, Furie BC, et al. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. *Nature*. 1992;**359**:848–51.
122. Huo Y, Weber C, Forlow SB, et al. The chemokine KC, but not monocyte chemoattractant protein-1, triggers monocyte arrest on early atherosclerotic endothelium. *J Clin Invest*. 2001;**108**:1307–14.
123. Huo Y, Schober A, Forlow SB, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med*. 2003;**9**:61–7.
124. Evangelista V, Manarini S, Dell'Elba G, et al. Clopidogrel inhibits platelet-leukocyte adhesion and platelet-dependent leukocyte activation. *Thromb Haemost*. 2005;**94**:568–77.
125. Steinhubl SR, Ellis SG, Wolski K, Lincoff AM, Topol EJ. Ticlopidine pretreatment before coronary stenting is associated with sustained decrease in adverse cardiac events: data from the Evaluation of Platelet IIb/IIIa Inhibitor for Stenting (EPISTENT) Trial. *Circulation*. 2001;**103**:1403–9.
126. Mehta SR, Yusuf S, Peters RJ, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet*. 2001;**358**:527–33.
127. Steinhubl SR, Berger PB, Mann 3rd JT, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *Jama*. 2002;**288**:2411–20.
128. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001;**345**:494–502.
129. Bhatt DL, Topol EJ. Scientific and therapeutic advances in antiplatelet therapy. *Nat Rev Drug Discov*. 2003;**2**:15–28.
130. Patrono C, Coller B, FitzGerald GA, Hirsh J, Roth G. Platelet-active drugs: the relationships among dose, effectiveness, and side effects: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest*. 2004;**126**:234S–64S.
131. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *Bmj*. 2002;**324**:71–86.

132. Patrono C, Garcia Rodriguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med*. 2005;**353**:2373—83.
133. Patrono C, Bachmann F, Baigent C, et al. Expert consensus document on the use of antiplatelet agents. The task force on the use of antiplatelet agents in patients with atherosclerotic cardiovascular disease of the European society of cardiology. *Eur Heart J*. 2004;**25**:166—81.
134. Belhassen L, Pelle G, Dubois-Rande JL, Adnot S. Improved endothelial function by the thromboxane A2 receptor antagonist S 18886 in patients with coronary artery disease treated with aspirin. *J Am Coll Cardiol*. 2003;**41**:1198—204.
135. Coller BS. Binding of abciximab to alpha V beta 3 and activated alpha M beta 2 receptors: with a review of platelet-leukocyte interactions. *Thromb Haemost*. 1999;**82**:326—36.
136. Straub A, Wendel HP, Azevedo R, Ziemer G. The GP IIb/IIIa inhibitor abciximab (ReoPro) decreases activation and interaction of platelets and leukocytes during in vitro cardiopulmonary bypass simulation. *Eur J Cardiothorac Surg*. 2005;**27**:617—21.
137. Randomized, placebo-controlled trial of titrated intravenous lamifiban for acute coronary syndromes. *Circulation*. 2002;**105**:316-321.
138. Boersma E, Harrington RA, Moliterno DJ, et al. Platelet glycoprotein IIb/IIIa inhibitors in acute coronary syndromes: a meta-analysis of all major randomised clinical trials. *Lancet*. 2002;**359**:189—98.
139. Chew DP, Bhatt DL, Sapp S, Topol EJ. Increased mortality with oral platelet glycoprotein IIb/IIIa antagonists: a meta-analysis of phase III multicenter randomized trials. *Circulation*. 2001;**103**:201—6.
140. Patrono C, Coller B, Dalen JE, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. *Chest*. 2001;**119**:39S—63S.