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## Platelets and Inflammation

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### INTRODUCTION

Platelets play a major physiological role in control of vascular integrity at the site of vascular lesions. However, the pathophysiological role of platelets is much broader than regulation of hemostasis and thrombosis. Platelets are critical elements in linking and modulating thrombosis, inflammation, and tissue repair. Platelets are stimulated by a variety of agonists including thrombin or ADP and also by inflammatory agents such as antibodies, complement, bacteria, and others. Platelets contribute to inflammation by interacting with inflammatory cells via adhesion and secretion of prestored proinflammatory mediators. Thus, platelets are critical elements in the pathophysiology of inflammation and modulate significantly a variety of inflammatory diseases. A profound understanding of the molecular mechanisms underlying the role of platelet in

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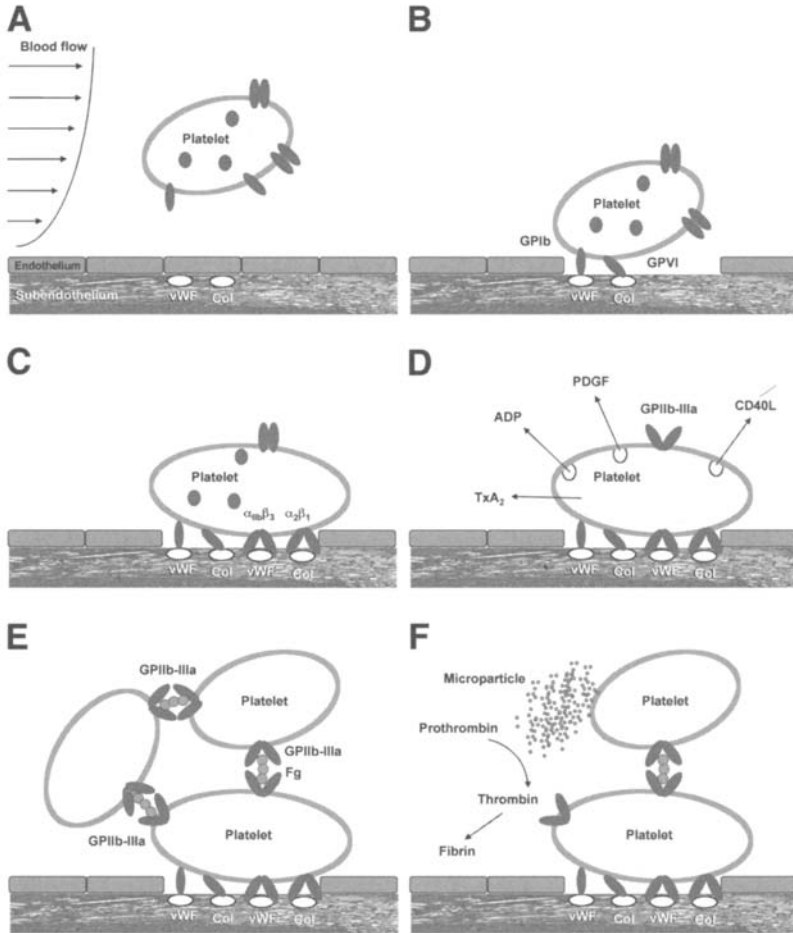
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inflammation may result in new therapeutic strategies in acute and chronic inflammatory diseases.

## THE PLATELET, THE INFLAMMATORY CELL

The fundamental role of platelets is to maintain the vascular integrity and to prevent loss of blood at sites of vascular injury. Under physiological conditions platelets circulate in the artery branch without interacting with each other or with structures of the intact vessel wall (1). At the location of an endothelial disruption, subendothelial matrix proteins including collagen and von Willebrand factor (vWF) are exposed to circulating platelets that are recognized by specific platelet membrane receptors such as glycoprotein (GP)VI (2) or the plasma membrane complex GPIIb-IX-V (3-5). Once adherent to the subendothelium, platelets spread on the surface and release granule-stored components that recruit additional platelets to the arterial lesion. In addition, adhering platelets express the activated form of the fibrinogen receptor GPIIb-IIIa that mediates platelet aggregation through formation of fibrinogen bridges between two adjacent platelets (6,7) (Fig. 1).

Despite regulation of hemostasis, platelets are involved in thrombosis and inflammation. Hemostasis, thrombosis, and inflammation are complex mechanisms with overlapping steps and connecting pathways. Interactions between platelets and inflammatory cells (e.g., endothelium, leukocytes) take place at various stages of the inflammatory process (8). They occur in the circulation as well as in extravascular inflammatory sites and are regulated through adhesion receptors and proinflammatory mediators that are released from internal stores of activated platelets. Platelets have been shown to be involved in inflammatory diseases caused by pathogens including bacteria, viruses, or parasites, leading to infectious endocarditis, sepsis, or the acquired thrombocytopenia that occurs during retroviral infections caused by the human immunodeficiency virus (HIV) and T-cell lymphotropic virus (9) (Table 1). In these diseases platelets participate in nonspecific immunodefense mechanisms caused by exogenous pathogens. Furthermore, platelets are involved in acute and chronic inflammation that is not directly related to infectious pathogens including diseases such as acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome, immune complex disease, allergic asthma, inflammatory bowel disease (10), multiple organ failure in septic shock (11,12), organ dysfunction associated with cardiopulmonary bypass (13), or acute coronary syndromes (14) including acute myocardial infarction and reperfusion (15,16) (Table 1). Moreover, platelets are fundamentally involved in chronic inflammatory



**Fig. 1.** (A) Platelet-dependent formation of a thrombus at an atherosclerotic plaque. Platelets do not adhere to the intact endothelial monolayer under physiological conditions. At the site of vascular lesions, extracellular matrix proteins like von Willebrand factor (vWF) and collagen (Col) are exposed to the blood. (B) Via the membrane adhesion receptors GPIb $\alpha$  and GPVI, platelets loosely adhere to the subendothelium. (C) This initial adhesion results in platelet activation and “opening” of the integrin receptors  $\alpha_{IIb}\beta_3$  (fibrinogen receptor) and  $\alpha_2\beta_1$  (collagen receptor). The interaction of  $\alpha_{IIb}\beta_3$  and  $\alpha_2\beta_1$  with extracellular matrix proteins stabilizes platelet adhesion (firm adhesion). (D) Subsequently, platelets spread and degranulate and thereby recruit additional platelets to the already adherent ones. (E) Platelets form microaggregates via fibrinogen “bridges” between two  $\alpha_{IIb}\beta_3$  receptors. (F) Formation of microparticles around the platelet aggregates catalyzes thrombin generation and thus fibrin formation that stabilizes the platelet thrombus. CD40L, CD40 ligand; PDGF, platelet-derived growth factor; TXA<sub>2</sub>, thromboxane A<sub>2</sub>.

**Table 1**  
**Role of Platelets in Inflammation**

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Inflammation caused by infection
Infectious endocarditis
Sepsis
Acquired idiopathic thrombocytopenia
Acute inflammation not directly related to infectious pathogens
Acute respiratory distress syndrome (ARDS)
Systemic inflammatory response syndrome (SIRS)
Multiple organ failure (MOF)
Immune complex disease
Allergic asthma
Cardiopulmonary bypass
Inflammatory bowel disease
Acute coronary syndrome (ACS)
Myocardial infarction and reperfusion
Chronic inflammation
Atherosclerosis
Development of vulnerable coronary plaque
Rheumatoid arthritis
Periodontitis

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diseases including atherosclerosis (17,18), progression, and induction of vulnerable coronary plaques leading to acute coronary syndrome (19), rheumatoid arthritis, or periodontitis (10) (Table 1). Thus, platelets are both target cell and effector in a variety of inflammatory diseases.

### RECEPTORS THAT REGULATE PLATELET ADHESION TO INFLAMMATORY CELLS

The mechanisms of thrombosis and inflammation require a close interaction among platelets, endothelium, leukocytes, plasma coagulation factors, and structures of the vessel wall (extracellular matrix) (20). Adhesion processes that are regulated by numerous adhesion receptors play a major role in these mechanisms. Platelets express multiple glycoproteins on their membranes that mediate the interactions with other platelets, with endothelial cells, or with leukocytes (21,22). Furthermore, platelet adhesion receptors mediate platelet attachment to subendothelial matrix proteins as well as interactions with plasmatic coagulation factors. Firm contact of platelets to cells or the vascular subendothelium enables platelets to localize a procoagulatory or inflammatory response in distinct locations of the vasculature (23).

**Table 2**  
**Membrane Glycoproteins Involved in Platelet Adhesion to Inflammatory Cells**

<i>Platelet membrane glycoprotein</i>	<i>Ligand/counterreceptor</i>
<b>Integrins</b>	
$\alpha_{IIb}\beta_3$ (GPIIb-IIIa, CD41-CD61)	Fg, Fn, Vn, vWF, Ln
$\alpha_v\beta_3$ (vitronectin receptor, CD51-CD61)	Vn, Fg, Fn
$\alpha_2\beta_1$ (collagen receptor, CD49b)	Coll, Ln
$\alpha_5\beta_1$ (fibronectin receptor, CD49c)	Fn
$\alpha_6\beta_1$ (laminin receptor, CD49f)	Ln
<b>Leucin-rich glycoproteins</b>	
GPIIb-V-IX (CD42a-b-c)	vWF, CD11b-CD18
GPIV (CD36)	TSP-1
<b>Selectins</b>	
P-selectin (CD62P)	PSGL-1
<b>Immunoglobulin-like adhesion receptors</b>	
ICAM-2	Fg, $\alpha_L\beta_2$ (LFA-1, CD11a)
PECAM-1	?
JAM-1	?
JAM-3	$\alpha_M\beta_2$ (MAC-1, CD11b)
GPVI	Coll

Abbreviations: GP, glycoprotein; Fg, fibrinogen; Vn, vitronectin; Fn, fibronectin; Coll, collagen; Ln, laminin; vWF, von Willebrand factor; TSP, thrombospondin; ICAM, intercellular adhesion molecule; PECAM, platelet-endothelial cell adhesion molecule; JAM, junctional adhesion molecule; LFA, leukocyte function-associated antigen; PSGL, P-selectin glycoprotein ligand.

Platelets express a variety of major membrane glycoproteins that are classified into four groups according to their characteristic structures: *integrins*, *leucine-rich glycoproteins*, *selectins*, and receptors of the *immunoglobulin-type* (24) (Table 2). All platelet membrane receptors have been shown to recognize a counterligand (e.g., fibrinogen) or receptor (e.g., P-selectin) on the partner cell and to mediate intercellular, heterotypic adhesion.

### *Integrins*

Integrins are adhesion receptors that link structures of the cytoskeleton to the extracellular matrix (25–27). Integrins are ubiquitous and can be found on almost all cells including platelets. In addition to platelet aggregation and adhesion, integrins participate in tissue development and differentiation as well as in the growth of atherosclerotic plaques and vascular remodeling. Integrins are noncovalently linked heterodimers

consisting of  $\alpha$ - and  $\beta$ -subunits. They are subdivided on the basis of the  $\beta$ -chain, which pairs with a specific  $\alpha$ -chain to form a functional receptor. To date 8  $\beta$ - and 14  $\alpha$ -subunits have been identified (25). The  $\beta$ -subunit serves for the structural classification, and the  $\alpha$ -subunit determines the specificity. Integrins interact with numerous glycoproteins (e.g., collagen, fibronectin, fibrinogen, laminin, thrombospondin, vitronectin, and vWF) (Table 2). These glycoproteins are components of the extracellular matrix, but they also occur in solution in plasma. The  $\beta_1$ - and  $\beta_3$ -integrins recognize the arginine-glycine-aspartate (RGD) amino acid sequence. The RGD sequence is a component of numerous extracellular matrix proteins and is also found in fibrinogen molecules. Synthetic peptides exhibiting the RGD sequence and having similar molecular structures are competitive inhibitors of ligand binding to integrins. Five different integrins have been described so far on platelets, three of the  $\beta_1$ -integrin class ( $\alpha_2\beta_1$  [collagen receptor],  $\alpha_5\beta_1$  [fibronectin receptor], and  $\alpha_6\beta_1$  [laminin receptor]) and two of the  $\beta_3$ -integrin family ( $\alpha_{IIb}\beta_3$  [fibrinogen receptor] and  $\alpha_v\beta_3$  [vitronectin receptor]) (25) (Table 2).

GPIIb-IIIa ( $\alpha_{IIb}\beta_3$ ) belongs to the  $\beta_3$ -integrins and is specific for the platelet/megakaryocyte system. GPIIb-IIIa is the most frequently occurring membrane glycoprotein of blood platelets and is constitutively expressed on the surface membrane. A substantial portion of GPIIb-IIIa is also present in intracellular stores ( $\alpha$ -granules and the open canalicular system) and can be translocated to the plasma membrane upon activation. The main task of GPIIb-IIIa is to bind fibrinogen to the activated platelet surface, which allows platelets to aggregate (homotype intercellular adhesion). Under physiological conditions, circulating platelets carry the resting, not activated GPIIb-IIIa complex on their surface (*low-affinity state*) (27). In the unactivated form, GPIIb-IIIa can only bind immobilized, but not soluble, fibrinogen. Platelet activation leads to a rapid change in the conformation of the GPIIb-IIIa complex and thereby to the exposure of high-affinity fibrinogen binding sites (*high-affinity state*) (27). This allows binding of soluble fibrinogen to the platelet surface and initiates aggregation. Besides its role in platelet aggregation, GPIIb-IIIa mediates platelet adhesion to subendothelial matrix proteins (fibrinogen, vWF, vitronectin, and laminin). Additionally, the receptor interferes with endothelial cells (heterotype intercellular adhesion) via the formation of fibrinogen "bridges" between the platelet GPIIb-IIIa and the vitronectin receptor  $\alpha_v\beta_3$  expressed on the luminal aspect of endothelial cells (27).

Whereas GPIIb-IIIa is specific for platelets and megakaryocytes, the vitronectin receptor ( $\alpha_v\beta_3$ ) is found on many different types of cells

including platelets, endothelial cells, and smooth muscle cells (7). Like GPIIb-IIIa ( $\alpha_{IIb}\beta_3$ ), the vitronectin receptor ( $\alpha_v\beta_3$ ) possesses the  $\beta_3$ -chain but this is paired with a different  $\alpha$ -chain ( $\alpha_v$ ) (7). In contrast to GPIIb-IIIa, the vitronectin receptor occurs in only very low amounts in platelets (50 receptors per platelet) and plays a very minor role in platelet function (7). The vitronectin receptor also binds other RGD-containing glycoproteins such as fibrinogen, fibronectin, vWF, and thrombospondin (Table 2).  $\alpha_v\beta_3$  contributes to the adhesion and migration of endothelial and smooth muscle cells to the extracellular matrix (7). Like GPIIb-IIIa,  $\alpha_v\beta_3$  possesses a binding site for the RGD sequence but not for the KQAGDV sequence. When  $\alpha_v\beta_3$ -positive cells adhere to specific matrix proteins, the receptors are enriched in the region of focal contact sites (clustering). The vitronectin receptor plays a role in angiogenesis, apoptosis, and proliferation (7,28). Inhibition of  $\alpha_v\beta_3$  by antagonists reduces the migration and proliferation of smooth muscle cells—mechanisms that participate in atherogenesis and intima proliferation.  $\alpha_v\beta_3$  is also expressed on the luminal side of endothelial cells and mediates the adhesion of activated platelets to endothelial cells. Besides the two  $\beta_3$ -integrins, platelets possess three  $\beta_1$ -integrins that seem to regulate the primary adhesion of platelets to the subendothelial matrix of the injured vessel wall. However, the exact role of  $\beta_1$ -integrins in hemostasis or thrombosis remains to be determined (7).

### *Leucine-Rich Glycoproteins*

Platelets contain two membrane glycoprotein complexes, GPIb-IX-V and GPIV, which are characterized by their abundance of the amino acid leucine (3–5). The GPIb-IX-V complex forms the adhesion receptor for vWF and plays a central role in primary hemostasis. The main task of GPIb-IX-V is the adhesion of circulating platelets to vWF immobilized in collagen fibrils in spite of the significant shear forces that exist in regions of arterial flow. The GPIb-IX-V complex consists of four subunits. GPIb $\alpha$  (150 kDa) and GPIb $\beta$  (27 kDa) are covalently linked to each other by disulfide bridges. Each GPIb molecule binds noncovalently to a GPIX molecule at a ratio of 1:1. GPV (82 kDa) is the central unit of the receptor complex and is surrounded by two GPIb and two GPIX proteins linked noncovalently with one another. All subunits of the GPIb-IX-V complex exhibit leucine-rich regions, whose functions are mostly unknown. The GPIb $\alpha$  subunit is very significant for the receptor function. In the region of the extracellular domain, GPIb $\alpha$  possesses binding sites for vWF and thrombin. The cytoplasmic domain anchors GPIb $\alpha$  in the cytoskeleton. Recent data indicate that GPIb $\alpha$  on platelets is a potential counterreceptor for Mac-1 (29). Furthermore, GPIb $\alpha$ -associated

vWF has also been proposed as a mediator of platelet-endothelium adhesion (30).

### *Selectins*

Selectins are vascular adhesion receptors that mediate the heterotype interactions of cells. The molecular structure of selectins is characterized by an extracellular lectin domain with a neighboring epidermal growth factor-like domain followed by short regions that are homologous to the regulatory complement factors (31,32). Three selectins have been described (31,32). E-selectin occurs on cytokine-activated endothelial cells and modulates granulocyte adhesion. Resting endothelial cells do not contain any E-selectin. L-selectin occurs constitutively on leukocytes, is shed from the leukocyte surface after activation, and participates in the adhesion of leukocytes to endothelial cells (31,32). P-selectin is found in platelets and endothelial cells, where it is stored in thrombocytic  $\alpha$ -granules and endothelial Weibel-Palade bodies, respectively. P-selectin is not expressed on the surface of resting platelets or endothelial cells. However, activation leads to the rapid release and surface expression of P-selectin on both types of cells. Both endothelial P-selectin and P-selectin exposed on activated adhering platelets at sites of endothelial disruption mediate initial tethering and rolling of leukocytes through their P-selectin glycoprotein ligand-1 (PSGL-1) (33,34). P-selectin binding to leukocytes induces activation of Mac-1 and allows firm adhesion and transplatelet migration (34).

### *Immunoglobulin-Type Receptors*

Several immunoglobulin-type receptors have been described on platelets including platelet-endothelial cell adhesion molecule-1 (PECAM-1) (6), intercellular adhesion molecule-2 (ICAM-2) (35), and junctional adhesion molecule-1 and -3 (JAM-1 and -3) (36–38). PECAM-1 occurs in platelets as well as in endothelial cells, neutrophils, and monocytes (6). The receptor appears to play a part in platelet adhesion to the subendothelium and to leukocytes by binding to glycosaminoglycans on their membrane. ICAM-2 has been described on activated platelets (35). The JAMs are newly discovered members of the Ig superfamily, which mediate cell-cell adhesion. Platelets contain at least two members of the JAM family including JAM-1 and JAM-3 (36). Platelet JAM-1 might be involved in primary hemostasis as the crosslinking of JAM-1 with Fc $\gamma$ RII resulted in platelet aggregation (27). It is also proposed to mediate platelet adhesion to cytokine-inflamed endothelial cells (39). Platelet JAM-3 has been recently shown to serve as a counterreceptor for Mac-1-mediated platelet-leukocyte interactions (38).



## PLATELET-DERIVED MEDIATORS THAT MODULATE FUNCTION OF INFLAMMATORY CELLS

Chemotaxis is a central mechanism to recruit inflammatory cells to a site of tissue damage or repair (40). Platelets exert an important influence on the chemotaxis of other cells. During adhesion, platelets are activated and release a variety of potent chemotactic factors that are either stored in their granules or cytoplasm or synthesized after stimulation (41,42). Moreover, platelets may by direct cell contact modulate the chemotactic properties of other cells including leukocytes (43,44) and endothelium (45,46). Platelets contain three different forms of storage granules: dense bodies or dense granules,  $\alpha$ -granules, and lysosomes. The granules are characteristic for platelets and serve as storage sites for proteins and other low-molecular-weight compounds (Table 3).

### *Dense Granules*

The dense granules are named after their characteristic electron-optical density and contain high levels of adenine and guanine nucleotides, divalent cations, and serotonin. Thus, during the release reaction, prothrombotic constituents are liberated that recruit other platelets to adhere and aggregate. Serotonin (5-hydroxytryptamine) acts predominantly as a local vasoconstrictor and proinflammatory compound. Serotonin has been proposed to enhance the chemotactic responsiveness of human leukocytes (47). Stimulation of monocytes through serotonin induces secretion of lymphocyte chemoattractant activity (interleukin-16 [IL-16]) that may promote the recruitment of T lymphocytes into an inflammatory focus (48,49). Other dense-granule constituents (histamine, ATP) have been shown to support the proinflammatory reaction in the microenvironment of platelet adhesion (41).

### *$\alpha$ -Granules*

$\alpha$ -Granules represent the major granule population in size and number (42). The  $\alpha$ -granules are typical secretory vesicles that carry proteins to the cell surface to be released. Some of the released intragranular proteins adhere to the platelet surface or become integrated into the plasma membrane, and some diffuse into the extracellular fluid (Table 3). Two specific platelet proteins,  $\beta$ -thromboglobulin ( $\beta$ -TG) and platelet factor-4 (PF-4) are localized in  $\alpha$ -granules together with proteoglycans (50). The latter include a family of  $\beta$ -TG-antigen molecules consisting of the platelet basic protein (PBP), connective tissue-activating protein-III (CTAP-III), and neutrophil-activating protein-2 (NAP-2), which are all precursors of  $\beta$ -TG and PF-4. Purified PF-4 lacks

Table 3  
Platelet-Derived Vasoactive, Proinflammatory, and Mitogenic Constituents

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<p>Dense granule</p> <ul style="list-style-type: none"> <li>Serotonin (5-HT)</li> <li>Adenine nucleotides: ATP, ADP</li> <li>Guanine nucleotides: GTP, GDP</li> <li>Histamine</li> </ul> <p><math>\alpha</math>-Granule</p> <ul style="list-style-type: none"> <li><math>\beta</math>-Tromboglobulin (<math>\beta</math>-TG), platelet factor-4 (PF-4)</li> <li>Platelet basic protein (PBP), connective tissue-activating protein III (CTAP-III), neutrophil-activating protein-2 (NAP-2)</li> <li>Epithelial neutrophil-activating protein-78 (ENA-78)</li> <li>Platelet-derived growth factor (PDGF)</li> <li>Transforming growth factor-<math>\beta</math> (TGF-<math>\beta</math>)</li> <li>Endothelial cell growth factor (ECGF)</li> <li>Epidermal growth factor (EGF)</li> <li>Vascular endothelial growth factor (VEGF)</li> <li>Insulin-like growth factor (IGF)</li> <li>Basic fibroblast growth factor (bFGF)</li> <li>Metalloproteinases 2 and 9</li> </ul> <p>Lysosome</p> <ul style="list-style-type: none"> <li>Acid proteases</li> <li>Glycohydrolases</li> </ul> <p>Cytosol</p> <ul style="list-style-type: none"> <li>Interleukin-1 <math>\beta</math> (IL-1 <math>\beta</math>)</li> <li>CD40 ligand (CD40L)</li> </ul> <p>Lipid mediators</p> <ul style="list-style-type: none"> <li>Platelet-activating factor (PAF)</li> <li>Thromboxane A<sub>2</sub> (TXA<sub>2</sub>)</li> <li>Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)</li> <li>Lysophosphatidic acid (LPA)</li> <li>Sphingosine-1 phosphate (SP-1)</li> </ul> <p>Microparticles</p>
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chemotactic activity for leukocytes but costimulation of leukocytes with tumor necrosis factor (TNF)- $\alpha$  results in exocytosis of secondary granule markers or tight adhesion to different surface proteins (51). PF-4 not only affects neutrophils, but it also induces the release of histamine by basophils and plays a role in eosinophil adhesion (42).  $\beta$ -TG represents a chemokine for neutrophils, monocytes, and lymphocytes.

Epithelial neutrophil-activating protein-78 (ENA-78) is another  $\alpha$ -granule constituent that stimulates neutrophil activation (52). Among the mitogenic factors present in platelet  $\alpha$ -granules, the specific platelet-

derived growth factor (PDGF) is present together with transforming growth factor- $\beta$  (TGF- $\beta$ ), endothelial cell growth factor (ECGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), vascular permeability factor (VPF), basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF) (52). PDGF and TGF- $\beta$  are growth factors that exert chemotactic properties on smooth muscle cells, macrophages, monocytes, and fibroblasts (52). Recently, PDGF and TGF- $\beta$  have been found to induce expression of VEGF in smooth muscle cells (53); VEGF is an endothelial mitogen and chemokine that stimulates endothelial cell migration. Furthermore, VEGF has been shown to support transendothelial migration of monocytes and to be chemotactic for mast cells, as well as monocytes. The chemokines RANTES and macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ) belong to the CC chemokines and are potent chemokines for basophils, eosinophils, T lymphocytes, and monocytes (52).

### *Lysosomes*

Lysosomes contain enzymes such as acid proteases and glycohydrolases that may participate in inflammation and extravasation of leukocytes through their cytotoxic and proteolytic activity at sites of platelet accumulation at inflamed tissue (54) (Table 3).

### *Cytosol*

Other important proinflammatory mediators such as IL-1 $\beta$  and CD40 ligand (CD40L) are present in the cytosol, are generated from mRNA, and are released upon activation (55). IL-1 $\beta$  is a central mediator in the cytokine cascade and is a potent activator of vascular cytokine production (55,57,58). CD40L is structurally related to TNF- $\alpha$  and translocates within seconds of activation to the platelet surface (46,56). CD40L stimulates endothelium to express ICAM-1, VCAM-1 and E-selectin, thus modulating leukocyte-endothelium interaction (46). Moreover, CD40L stimulates platelet secretion and stabilizes platelet aggregation by interference with GPIIb-IIIa (59).

### *Lipid Mediators*

In addition to granule- and cytoplasm-stored substances, platelets release lipid mediators upon activation including platelet-activating factor (PAF), thromboxane A<sub>2</sub> (TXA<sub>2</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), platelet-derived lysophosphatidic acid (LPA), and sphingosine-1-phosphate (SP-1) (60). PAF is a phosphoglyceride generated from the phospholipids of cell membranes. PAF promotes leukocyte-endothelium interaction and favors diapedesis of leukocytes (61). TXA<sub>2</sub> is another potent

platelet mediator derived from the platelet membrane. Upon platelet activation TXA<sub>2</sub> is synthesized *de novo* from arachidonic acid through the action of platelet cyclooxygenase-1 (COX-1), an enzyme that is inhibited irreversibly by aspirin (62). TXA<sub>2</sub> is known to induce platelet aggregation at sites of platelet activation. In addition, the arachidonic acid metabolite acts as a potent proinflammatory mediator, inducing leukocyte adhesion to and migration across activated endothelial cells (62). Another rich source of proinflammatory arachidonic acid metabolites is the platelet microparticles, membrane blebs that are shed from the surface of activated platelets by membrane vesiculation (63). Platelet microparticles have been shown to increase the adhesion of monocytes to endothelial cells in a time- and dose-dependent manner (63). In line with this finding, microparticle-derived arachidonic acid enhanced the expression of ICAM-1 on resting endothelial cells and upregulated CD11a/CD18 as well as CD11b/CD18 on monocytes.

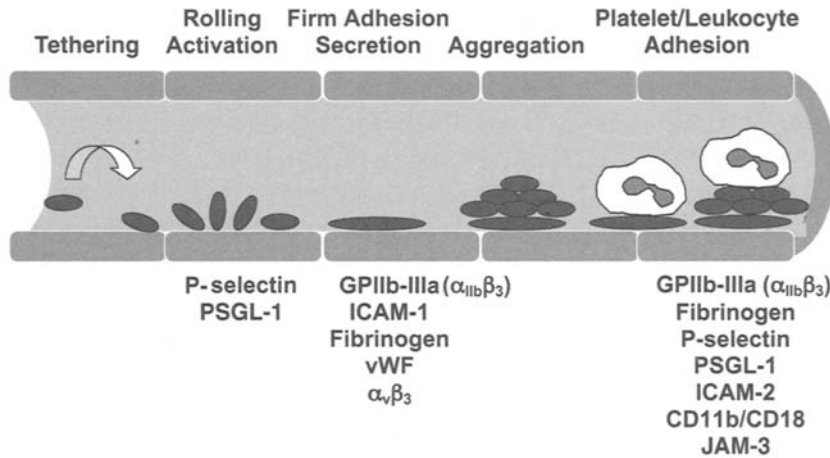
Another potent inflammatory mediator, which is released in high amounts by activated platelets, is the cellular phospholipid LPA (64). LPA also leads to the activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B) in endothelial cells, induces the upregulation of endothelial adhesion molecules, such as E-selectin and ICAM-1, and initiates the release of MCP-1 and IL-8 (64). Hence, LPA released during platelet-endothelial cell interactions at sites of endothelial dysfunction could function in a paracrine manner, directly modulating inflammatory and proliferative responses in the vascular wall.

## PLATELET INTERACTION WITH THE ENDOTHELIUM

The vascular endothelium is central for maintaining homeostasis. Endothelial cells produce and present molecules that define the inflammatory properties of the vessel wall. They have dynamic control over chemotaxis, adhesion, and extravasation of circulating leukocytes to areas of injury or inflammation (65). Control mechanisms include the expression and release of cytokines and chemokines and cell surface presentation of adhesion molecules in response to a diverse set of environmental elements, including rheologic and oxidative stress, cytokines, hormones, and infection.

### *Platelet Adhesion to Endothelium*

In the past, numerous studies have shown that platelets can adhere to the intact endothelial monolayer and substantially modulate endothelial cell function (67–75). Thus, under certain pathophysiological circumstances, endothelial denudation and exposure of subendothelial matrix



**Fig. 2.** Platelet adhesion to the endothelium. ICAM, intercellular adhesion molecule; JAM, junctional adhesion molecule; PSGL, P-selectin glycoprotein ligand; vWF, von Willebrand factor.

are not required for platelet adhesion to the vascular wall. Adherent platelets release a variety of proinflammatory mediators and growth hormones and have the potential to modify signaling cascades in vascular cells, inducing the expression of endothelial adhesion receptors and the release of endothelial chemoattractants. In this manner they might regulate the adhesion and infiltration of leukocytes, in particular of monocytes, into the vascular wall, a process thought to play a key role in acute and chronic inflammation. Normal “resting” endothelium represents a nonadhesive and nonthrombogenic surface that prevents extravasation of circulating blood cells (66). In contrast, activated endothelial cells are proadhesive and promote the adhesion of circulating blood platelets. Adhesion of platelets to the intact but activated endothelium in the absence of previous endothelial denudation involves a surface receptor-dependent process that allows “capturing” of circulating platelets toward the vessel wall even under high shear stress. Similar to the recruitment of leukocytes (65), the adhesion of platelets to the vascular endothelial surface is a multistep process, in which platelets are tethered to the vascular wall, followed by platelet rolling and subsequent firm adhesion (Fig. 2).

Whereas the adhesion receptors involved in platelet attachment to the subendothelial matrix, e.g., following rupture of an atherosclerotic plaque, have been well defined during the past decade (Fig. 1), few studies have focused on the molecular determinants that promote the

interaction between platelets and the intact vascular endothelium. The initial loose contact between circulating platelets and vascular endothelium (*platelet rolling*) is mediated by selectins, present on both endothelial cells and platelets (76–80). P-selectin (CD62P) is rapidly expressed on the endothelial surface in response to inflammatory stimuli by translocating from membranes of storage granules (Weibel-Palade bodies) to the plasma membrane within seconds. Endothelial P-selectin has been demonstrated to mediate platelet rolling in both arterioles and venules in acute inflammatory processes (76,79). E-selectin, which is also expressed on inflamed endothelial cells, also allows a loose contact between platelets and endothelium *in vivo* (80). In line with the concept of endothelial inflammation as a trigger for platelet accumulation, the process of platelet rolling does not require previous platelet activation, since platelets from mice lacking P- and/or E-selectin roll as efficiently as wild-type platelets (81).

So far few studies have addressed the exact nature of the ligands expressed on platelets that bind to endothelial P- or E-selectin. PSGL-1, a glycoprotein that avidly associates with P-selectin, is present predominantly on myeloid cells and mediates leukocyte-endothelium interactions and leukocyte-platelet interactions *in vitro* and *in vivo* (82,83).

Another sialomucin that has been identified as a potential counter-receptor for platelet P-selectin is the leucine-rich GPIb-IX-V, the vWF receptor complex. Romo et al. (30) have demonstrated recently that cells expressing P-selectin roll on immobilized GPIb $\alpha$ . Platelet rolling on activated endothelium can be inhibited by antibodies against both P-selectin and GPIb $\alpha$ , indicating that the vWF receptor mediates platelet adhesion both to the subendothelial matrix and to intact endothelial cells. Future studies are needed to clarify whether the association of GPIb $\alpha$  with P-selectin leads to platelet activation similar to GPIb $\alpha$ -vWF interaction.

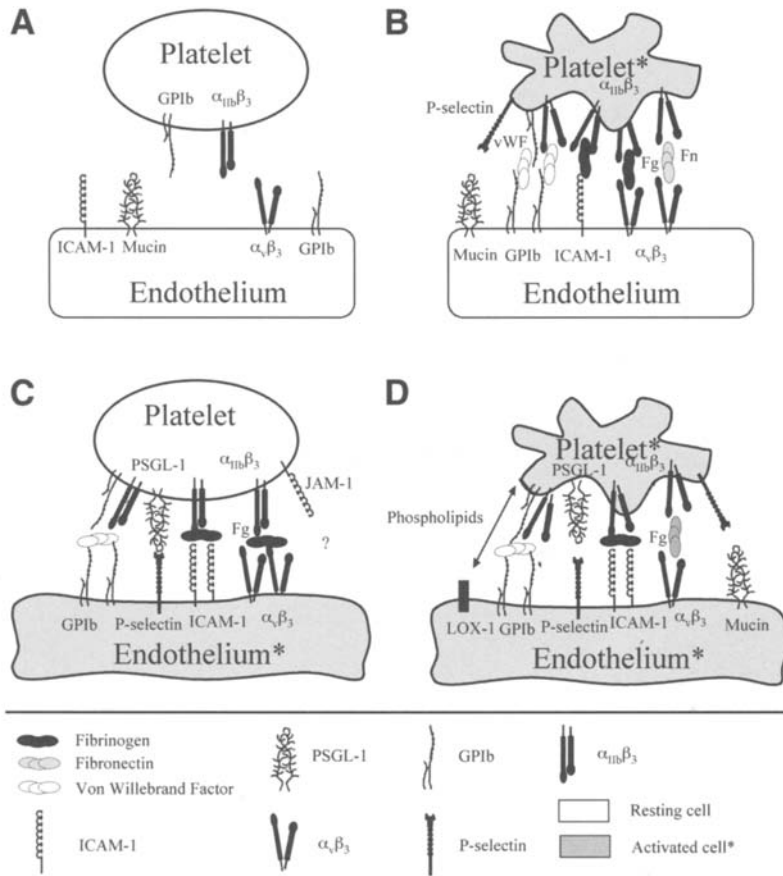
Interactions of selectins with their counterreceptors are characterized by high on and off rates, enabling platelets to attach rapidly to the endothelial monolayer with high resistance to tensile stress, explaining why adherent platelets can oppose the drag created by the shear rates present particularly in arterioles. On the other hand, these bonds have an intrinsically high dissociation rate and thus a limited half-life, which results in detachment at the trailing edge of platelets, where the tension is greatest, resulting in forward rotational movement (*rolling*) from torque imposed by the blood flow. However, because of their biophysical characteristics, selectin-ligand interactions are not sufficient to promote firm adhesion of platelets in the blood stream. This indicates that during rotational movement, new bonds, characterized by low dissociation rates, have to be formed that promote irreversible adhesion. These tighter

interactions between platelets and the vascular wall involve the interplay of platelets and endothelial integrins as well as immunoglobulin-like adhesion molecules.

Apart from leucine-rich glycoproteins, the integrins are the major group of receptors involved in mediating platelet adhesion to matrix proteins including collagen, vitronectin, fibronectin, and laminin. GPIIb-IIIa ( $\alpha_{IIb}\beta_3$ ) is the major integrin on platelets and plays a key role in platelet accumulation on activated endothelium. In the presence of soluble fibrinogen,  $\alpha_{IIb}\beta_3$  mediates heterotypic cell adhesion to  $\alpha_v\beta_3$ -expressing cells including endothelial cells (16,84). Moreover, platelets firmly adhere to activated endothelial cells via  $\alpha_{IIb}\beta_3$ , a mechanism that can be blocked by antagonists of  $\beta_3$ -integrins (16). In vivo, firm platelet adhesion to the endothelium can be inhibited by anti- $\alpha_{IIb}\beta_3$  monoclonal antibody and platelets defective in  $\alpha_{IIb}\beta_3$  do not adhere firmly to activated endothelial cells (85). Taken together, these data indicate that, apart from mediating platelet aggregation, the platelet fibrinogen receptor is of paramount importance in mediating firm attachment of platelets to the vascular endothelium (Fig. 2).

Only a few integrins have been reported to be expressed on the luminal side of endothelial cells. Among them, the vitronectin receptor ( $\alpha_v\beta_3$ ) appears to play a crucial role in promoting platelet adhesion. The vitronectin receptor is upregulated in response to endothelial cell activation, e.g., by IL-1 $\beta$  or thrombin (16,86). Inhibition of  $\alpha_v\beta_3$  attenuates platelet-endothelial cell interaction (16). Hence, both platelet  $\alpha_{IIb}\beta_3$  and endothelial  $\alpha_v\beta_3$  are involved in mediating firm adhesion of platelets to the luminal aspect of activated endothelial cells. However, direct binding of  $\alpha_{IIb}\beta_3$  to endothelial  $\alpha_v\beta_3$  has not been reported so far. In fact, heterotypic cell adhesion through  $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$  requires the presence of fibrinogen, which bridges the platelet fibrinogen receptor to the endothelial vitronectin receptor (84). Recent evidence suggests that this fibrinogen-dependent bridging mechanism rather than direct receptor-ligand interactions mediates firm platelet adhesion to the endothelium both in vitro and in vivo (Fig. 3) (85). The affinity of the platelet  $\alpha_{IIb}\beta_3$  for its ligand underlies strict regulation and increases with platelet activation (inside out-integrin signaling; see p.120). Although even nonactivated  $\alpha_{IIb}\beta_3$  can bind to immobilized fibrinogen, platelet activation during the initial contact between platelets and endothelial cells (platelet rolling), e.g., via GPIIb $\alpha$ -P-selectin interaction, might enhance the fibrinogen binding capacity of  $\alpha_{IIb}\beta_3$  and thereby facilitate subsequent firm platelet adhesion (30).

Recently, two members of the JAM adhesion receptor family have been identified in platelets, JAM-1 and JAM-3 (36–39). Platelets constitutively express JAM-1 (F11R) and inhibit JAM-1 by blocking mono-

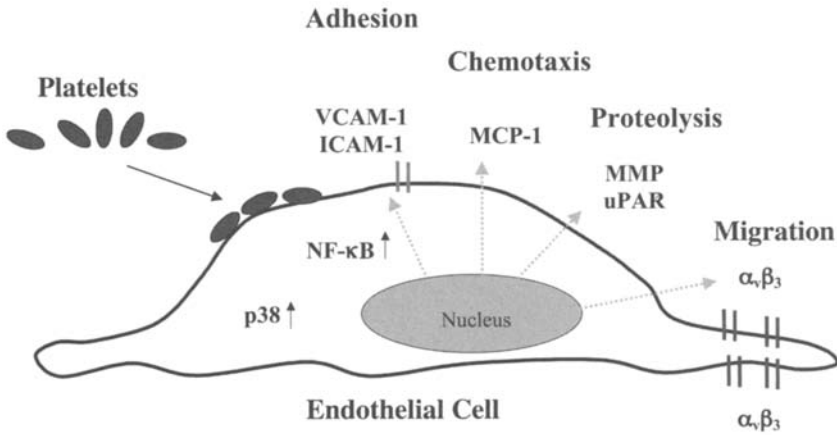


**Fig. 3.** Various possibilities of platelet interaction with endothelial cells. (A) Resting platelet and resting endothelium; (B) activated platelet and resting endothelium; (C) resting platelet and activated endothelium; (D) both activated platelet and endothelium. Fg, fibrinogen; Fn, fibronectin, ICAM, intercellular adhesion molecule; JAM, junctional adhesion molecule; LOX-1, lectin-like oxidized low-density lipoprotein; PSGL, P-selectin glycoprotein ligand; vWF, von Willebrand factor.

clonal antibody-reduced platelet adhesion to TNF- $\alpha$ -treated endothelial cells (Fig. 3).

Apart from the traditional adhesion receptors, *scavenger receptors* that bind oxidized low-density lipoprotein (oxLDL) appear to be involved in the recruitment of blood cells to the inflamed endothelial surface. The endothelial receptor for oxLDL, lectin-like oxLDL-1 (LOX-1), has been proposed to promote platelet adhesion to the endothelial cell surface (74). Phosphatidylserines, exposed on the surface of activated





**Fig. 4.** Platelet-induced endothelial inflammation. ICAM, intercellular adhesion molecule; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB, uPAR, protease-activated receptor; VCAM, vascular cell adhesion molecule.

platelets, are the ligands for LOX-1. Since LOX-1 expression on endothelial cells is upregulated *in vivo* by hypertension and hypercholesterolemia, the scavenger receptor might be important in initiating platelet adhesion to dysfunctional endothelium, especially in the early process of atherosclerosis (Fig. 3).

In summary, platelet-endothelial cell interactions are a multistep process, in which selectins and integrins or immunoglobulin-like adhesion receptors play a predominant role. These receptor-dependent platelet-endothelial cell interactions allow transcellular communication via soluble mediators and might therefore play an important role in the initiation and progression of vascular inflammation (Fig. 3).

### *Platelet-Induced Endothelial Activation*

During the adhesion process platelets are activated and release an arsenal of potent proinflammatory and promitogenic substances into the local microenvironment, thereby altering chemotactic, adhesive, and proteolytic properties of endothelial cells (Fig. 4). These platelet-induced alterations of the endothelial phenotype support chemotaxis, adhesion, and transmigration of monocytes to the site of inflammation. Among the various platelet-derived proinflammatory proteins, IL-1β has been identified as a major mediator of platelet-induced activation of endothelial cells (57,58). The IL-1β activity expressed by platelets appears to be associated with the platelet surface (57,58), and cocubation of endothelial cells with thrombin-activated platelets induces IL-1β-

dependent secretion of IL-6 and IL-8 from endothelial cells. Furthermore, incubation of cultured endothelial cells with thrombin-stimulated platelets significantly enhances the secretion of endothelial monocyte chemoattractant protein-1 (MCP-1) in an IL-1 $\beta$ -dependent manner (86,91). MCP-1 belongs to the CC family of chemokines and is thought to play a key role in the regulation of monocyte recruitment to inflamed tissue (91).

However, platelet IL-1 $\beta$  does not only modify endothelial release of chemotactic proteins, it also has the potential to increase endothelial expression of adhesion molecules. Surface expression of intercellular adhesion molecule-1 (ICAM-1) and  $\alpha_v\beta_3$  on endothelial cells is significantly enhanced by activated platelets via IL-1 $\beta$  (86). Both enhanced chemokine release and upregulation of endothelial adhesion molecules through platelet-derived IL-1 $\beta$  act in concert and promote neutrophil and monocyte adhesion to the endothelium. IL-1 $\beta$ -dependent expression of early inflammatory genes, such as MCP-1 or ICAM-1, involves the activation of the transcription factor NF- $\kappa$ B. Transient adhesion of platelets to the endothelium initiates degradation of inhibitor of  $\kappa$ B (I $\kappa$ B) and supports activation of NF- $\kappa$ B in endothelial cells, thereby inducing NF- $\kappa$ B-dependent chemokine gene transcription (92,93). Parallel to this finding, transfection of "decoy"  $\kappa$ B oligonucleotides or a dominant-negative I $\kappa$ B kinase (IKK) mutant attenuates platelet-induced nuclear translocation of NF- $\kappa$ B and MCP-1 secretion in endothelial cells (93). Likewise, platelet-induced NF- $\kappa$ B-activation was found to be largely reduced by IL-1 $\beta$  antagonists, supporting the notion that platelet IL-1 $\beta$  is the molecular determinant of platelet-dependent activation of the transcription factor.

Activation of NF- $\kappa$ B involves a cascade of phosphorylation processes. One family of kinases that is involved in NF- $\kappa$ B-dependent gene expression is the mitogen-activated protein (MAP) kinases, such as p38 MAP kinase. In a manner similar to recombinant human IL-1 $\beta$ , activated platelets have the potential to induce phosphorylation of p38 MAP kinase. Correspondingly, transfection of a dominant-negative p38 mutant significantly reduced platelet-induced MCP-1 secretion in endothelial cells (94). Taken together, these data indicate that platelet-derived IL-1 $\beta$  initiates NF- $\kappa$ B dependent expression of chemotactic and adhesive proteins in endothelial cells. In this manner, platelets promote the recruitment of both neutrophils and monocytes to the endothelial cell surface, thus inducing inflammation.

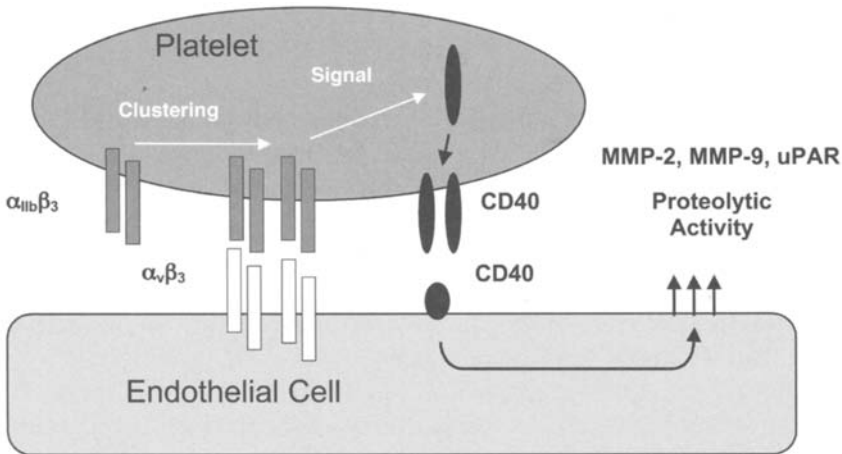
Another platelet-derived chemokine is the regulated on activation, T-cell expressed and secreted chemokine (RANTES), which has been seen to trigger monocyte arrest on inflamed and atherosclerotic endothelium

(95). Deposition of platelet RANTES induces monocyte recruitment mediated by P-selectin (96). Furthermore, release of platelet-derived CD40 ligand induces inflammatory responses in endothelium. CD154 (CD40L), a 30–33-kDa protein, belongs to the TNF family of cytokines, which includes TNF- $\alpha$  and Fas ligand. CD40L was originally thought to be restricted to CD4<sup>+</sup> T lymphocytes, mast cells, and basophils. Henn et al. (46) showed that platelets store CD40L in high amounts and release CD40L within seconds following activation *in vitro*. Ligation of CD40 on endothelial cells by CD40L expressed on the surface of activated platelets increased the release of IL-8 and MCP-1, the principal chemoattractants for neutrophils and monocytes (46). In addition, platelet CD40L enhanced the expression of endothelial adhesion receptors including E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and ICAM-1, all molecules that mediate the attachment of neutrophils, monocytes, and lymphocytes to the inflamed vessel wall. Moreover, CD40L induces endothelial tissue factor expression (97). Hence, like IL-1 $\beta$ , CD40L expressed on platelets induces endothelial cells to release chemokines and to express adhesion molecules, thereby generating signals for the recruitment of leukocytes in the process of inflammation.

CD40 ligation on endothelial cells, smooth muscle cells, and macrophages initiates the expression and release of matrix-degrading enzymes, the matrix metalloproteinases (MMPs). These enzymes, which degrade extracellular matrix proteins, contribute significantly to the destruction and remodeling of inflamed tissue. Activated platelets release MMP-2 and MMP-9 during aggregation (98). Moreover, adhesion of activated platelets to endothelial cells results in generation and secretion of MMP-9 and of the protease-activated receptor uPAR on cultured endothelium (99). The endothelial release of MMP-9 was dependent on both the fibrinogen receptor GPIIb-IIIa and CD40L because inhibition of either mechanism resulted in reduction of platelet-induced matrix degradation activity of endothelial cells. Moreover, GPIIb-IIIa ligation results in substantial release of CD40L in the absence of any further platelet agonist (100) (Fig. 5). These results suggest that the release of platelet-derived proinflammatory mediators like CD40L is dependent on GPIIb-IIIa-mediated adhesion. This mechanism may be pathophysiologically important for the localization of platelet-induced inflammation of the endothelium at a site of platelet-endothelium adhesion.

## PLATELET INTERACTION WITH LEUKOCYTES

Platelet adhesion to the endothelium or the subendothelial matrix induces platelet activation and the release of substances that are able to cause chemotaxis and migration of circulating leukocytes toward the site

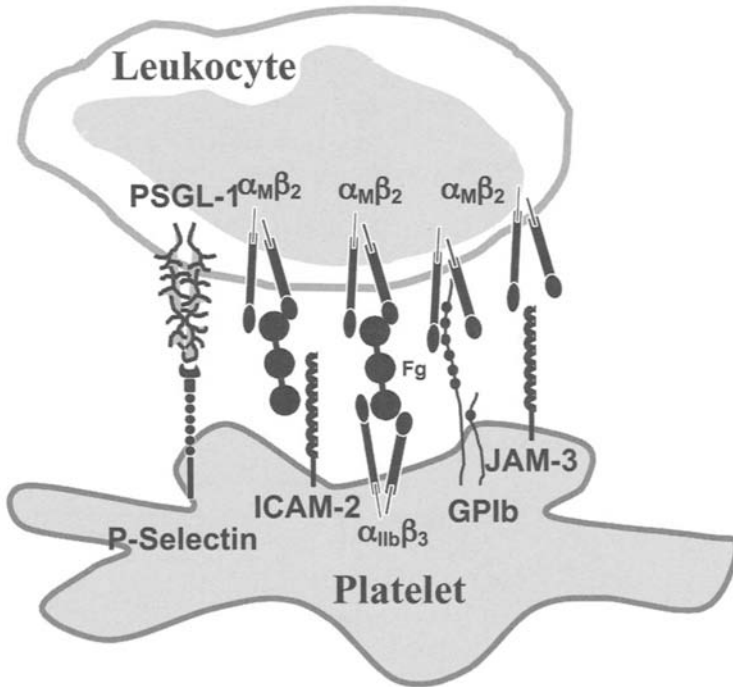


**Fig. 5.** GPIIb-IIIa-mediated release of CD40 ligand (CD40L) and pericellular proteolysis. MMP, matrix metalloproteinase; uPAR, protease-activated receptor.

of platelets accumulation. Platelets and leukocytes can form heterotypic conjugates, i.e., platelet-leukocyte aggregates, both *in vivo* and *in vitro* (101). All leukocytes subpopulations can conjugate platelets (102,103). Among them, monocytes show the greatest, and lymphocytes the least, propensity to form aggregates with platelets. Accumulating evidence indicates that close cell–cell contact mutually regulates platelet and leukocyte functions. Leukocyte-bound platelets may facilitate leukocyte rolling, adhesion, and migration on/through the vessel wall and thus enhance the accumulation of leukocytes at inflammatory sites (104). For example, neutrophil adhesion to cultured endothelial cells, or neutrophil adhesion to damaged arterial segments is enhanced in the presence of platelets (104). Platelet-derived microparticles may also enhance monocyte adhesion to the endothelium (63). Similarly, leukocytes can enhance platelet adhesion to endothelial cells by platelet-leukocyte aggregation. Activated platelets may facilitate leukocyte recruitment into a growing thrombus; leukocytes, in turn, contribute to platelet activation and fibrin deposition (105).

### ***Mechanisms of Platelet Adhesion to Leukocytes***

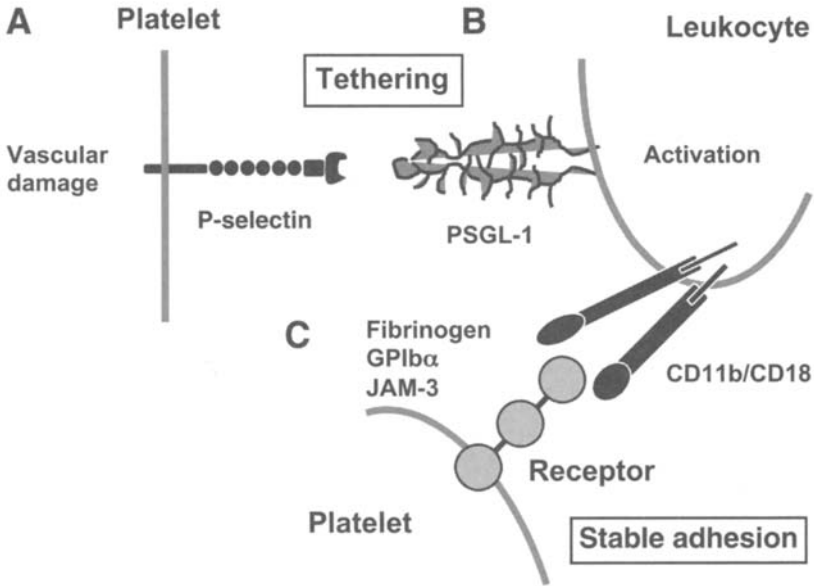
Like platelet adhesion to the vessel wall, leukocyte recruitment to the vascular endothelium requires multistep adhesive and signaling events including selectin-mediated rolling, leukocyte activation, and integrin-mediated firm adhesion and diapedesis (65) (Fig. 6). Several ligand-receptor systems have been found to be involved in platelet-leukocyte aggregation (Fig. 6). Platelet-expressed P-selectin (106,107) and leuko-



**Fig. 6.** Receptors that mediate platelet adhesion to leukocytes. ICAM, intercellular adhesion molecule; JAM, junctional adhesion molecule; PSGL, P-selectin glycoprotein ligand.

cyte-expressed PSGL-1/CD15 are the most important receptors for platelet-leukocyte adhesion. The bridging of platelet GPIIb-IIIa and leukocyte  $\beta_2$ -integrins (CD11b/CD18 and CD11c/CD18) by fibrinogen (108) and the ligation of CD36 (on both cell types) via thrombospondin (109) also contribute to platelet-leukocyte adhesion. Furthermore, CD40/CD40L (108, 109), ICAM-1/ $\beta_2$ -integrins (87), GPIb $\alpha$ /Mac-1, and JAM-3/Mac-1 (38) are also involved in platelet-leukocyte adhesion. At sites of platelet adhesion to the endothelium or subendothelium, leukocyte infiltration can also occur through interactions with platelets and fibrin (105).

Platelets either immobilized on a surface or activated in suspension express a complete machinery to recruit leukocytes, as follows: (1) platelet P-selectin is a mediator of the first contact (tethering); (2) interaction of platelet P-selectin with its counterreceptor PSGL-1 on leukocytes induces signaling events relevant for Mac-1 activation; and (3) the activated  $\beta_2$ -integrin (Mac-1) on leukocytes allows and reinforces firm platelet-leukocyte adhesion through binding to counterreceptors (ICAM-



**Fig. 7.** Platelet-induced activation of leukocytes via P-selectin. (A) Platelet tethering to leukocytes via P-selectin/PSGL-1 adhesion. (B) P-selectin/PSGL-1-mediated leukocyte activation. (C) Consolidation of platelet-leukocyte adhesion via interaction of MAC-1 (CD11b/CD18) with platelet-bound fibrinogen or platelet surface-expressed GPIIb or junctional adhesion molecule-3 (JAM-3). PSGL, P-selectin glycoprotein ligand.

2, fibrinogen bound to GPIIb-III $\alpha$ , GPIIb $\alpha$ , JAM-3) present on the platelet surface (Fig. 7).

Platelet adhesion to leukocytes enhances the proinflammatory and prothrombotic activities of leukocytes (112). Thus, leukocyte-bound platelets are known to enhance leukocyte adhesion molecule expression, superoxide anion generation, tissue factor expression, and cytokine synthesis (43,44,113,114). These effects seem to be closely regulated by intercellular signaling via adhesion molecule ligation. PSGL-1/P-selectin ligation induces a tyrosine-kinase-dependent signal, which upregulates Mac-1 expression and enhances  $\beta_2$ -integrin-dependent neutrophil adhesion to ICAM-1 (115). P-selectin/PSGL-1 interaction markedly enhances tissue factor activity in monocytes, and PSGL-1 blockade can inhibit tissue factor expression (116,117). Furthermore, PSGL-1 engagement also mobilizes transcription factor NF- $\kappa$ B and induces cytokine expression in monocytes (43). Signaling via  $\beta_2$ -integrins is necessary for neutrophil oxidative bursts, and platelets conjugating to neutrophil  $\beta_2$ -integrin via fibrinogen upregulate neutrophil oxidative burst activity (118).  $\beta_2$ -integrin is also involved in the expression of  $\beta_1$ -

integrins ( $\alpha_4\beta_1$  and  $\alpha_2\beta_1$ ), which are important in neutrophil adhesion and migration (119) (Fig. 7).

### ***Platelet-Induced Leukocyte Activation Via Soluble Mediators***

Apart from direct cell-cell conjugation, leukocyte function can be influenced by platelet-derived soluble mediators. A number of proteins stored in platelet  $\alpha$ -granules have been shown to enhance leukocyte activation (Table 2). Platelets contain large amounts of two chemokines, PF-4 and  $\beta$ -TG, which are stored in  $\alpha$ -granules. PF-4 is chemotactic for neutrophils and monocytes and can induce leukocyte adhesion to endothelial cells and degranulation via activation of leukocyte function-associated antigen-1 (LFA-1) (120).  $\beta$ -TG is the precursor of NAP-2, which stimulates neutrophil chemotaxis, adherence, calcium mobilization, and degranulation of lysosomes (120,121). It is interesting to note that neutrophil-derived cathepsin G is the principal enzyme cleaving  $\beta$ -thromboglobulin into NAP-2, and that this product then acts on neutrophils themselves (122–125). Platelet  $\alpha$ -granules also contain PDGF, which may enhance leukocyte adhesion, degranulation, chemotaxis, phagocytosis, and tissue factor expression (126–128). Moreover, platelet-released fibrinogen may induce oxidative bursts in neutrophils by bridging GPIIb-IIIa and CD11c/CD18 (129).

Small molecules stored in platelet dense granules also regulate various aspects of leukocyte function. Adenine nucleotides (ATP and ADP) can induce adhesion, aggregation, and degranulation of neutrophils and monocytes. Adenine and guanine nucleotides have also been shown to evoke superoxide anion production in neutrophils (130). Serotonin may enhance leukocyte rolling and adhesion by upregulating the expression of leukocyte adhesion molecules, such as Mac-1. Activated leukocytes generate PAF, which induces platelet aggregation, platelet adhesion to endothelial cells, and platelet secretion. Platelet-released chemokines, e.g., RANTES, have been reported to induce monocyte adhesion on endothelial cells (95). Other soluble components released from platelet membranes may also activate leukocytes. For instance, soluble CD40L may bind to monocyte CD40 and promote tissue factor expression and cytokine/chemokine production (97,99). Recombinant P-selectin has also been shown to enhance neutrophil phagocytosis by influencing  $\beta_2$ -integrin function (112).

## **PLATELETS AND ATHEROSCLEROSIS**

It is increasingly recognized that atherosclerosis is a chronic inflammatory condition that can be converted into an acute clinical event by atherosclerotic plaque progression, plaque rupture, and thrombosis

(131). Platelets play a major role in atherogenesis and thrombotic events in diseased arteries. Healthy endothelium maintains vascular integrity and provides a nonthrombotic inner layer of the arterial wall. Chronic endothelial injury and inflammation are key features in the pathogenesis of atherosclerosis (131). Cardiovascular risk factors such as hypercholesterolemia, hypertension, smoking, and diabetes result in oxidative stress of the vascular wall, endothelial dysfunction, and platelet hyperactivity (131). Atherosclerosis emerges locally and preferentially at lesion-prone sites (132) that are characterized by decreased shear stress because of nonlaminar, turbulent blood flow at branching vessels or curves. Normal arterial shear stress is atheroprotective, with high expression of nitric oxide synthetase and COX, whereas decreased shear stress is associated with an atherogenic endothelial phenotype, which expresses ICAM-1, MCP-1, and PDGF (132). Several indications suggest that platelets significantly contribute to the inflammatory processes that promote atherosclerotic lesion formation.

Platelets play a key role in thrombotic vascular occlusion at the atherosclerotic plaque leading to acute thrombotic episodes that result in acute coronary syndrome and stroke (1). After the prolonged process of silent plaque evolution, atheromas become susceptible to plaque rupture, leading to platelet adhesion and subsequent activation on the exposed, highly thrombogenic subendothelial surface, which initiates the dreaded clinical complications of thromboischemic episodes (133,134). Enhanced responsiveness of the atherosclerotic plaque to platelets can be triggered, for example, by changes in the shear forces supporting platelet recruitment to the vulnerable plaque. Furthermore, biological changes in the form of chemotaxis of monocytes toward the plaque region, transmigration through the endothelial barrier, macrophage differentiation, phagocytosis, and secretion of proteolytic enzymes (plasminogen activator system, metalloproteinases) appear to contribute substantially to the instability of the plaque.

Over the past few years platelets have been recognized to play a critical role in the chemotaxis and migration of monocytes, thus promoting inflammation and progression of atherosclerosis. Experimental evidence indicates that platelets might contribute significantly to the inflammatory processes that promote atherosclerotic lesion formation. Platelets can adhere directly to the intact endothelial monolayer even in the absence of endothelial disruption (135). During adhesion platelets are activated and release proinflammatory cytokines and chemoattractants (such as IL-1 $\beta$ , PF-4, and CD40 ligand). In vitro interactions between platelets and endothelial cells trigger the secretion of chemokines and the expression of adhesion molecules that promote the

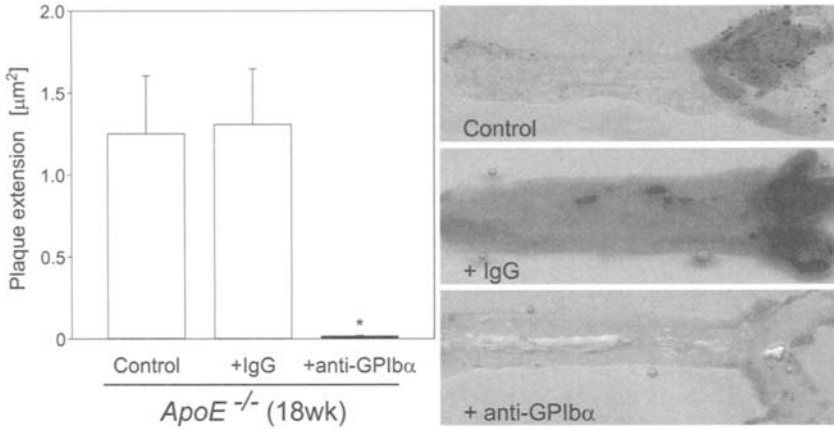


adhesion of leukocytes (Fig. 4). In this manner, the adhesion of platelets to the endothelial surface might generate signals that lead to the recruitment and extravasation of monocytes during atherosclerotic plaque progression, a process of paramount importance for atherogenesis. Moreover, platelets have been found to alter substantially the pericellular proteolytic activity of endothelial cells (activation of metalloproteinases), resulting in extracellular matrix degradation and fibrous cap thinning of a vulnerable lesion (Fig. 5) (98). Thus, in areas of platelet accumulation at lesion-prone sites, an enhanced release of platelet-derived proatherogenic compounds favors atherogenetic changes within the arterial wall and the development of atherosclerotic plaques.

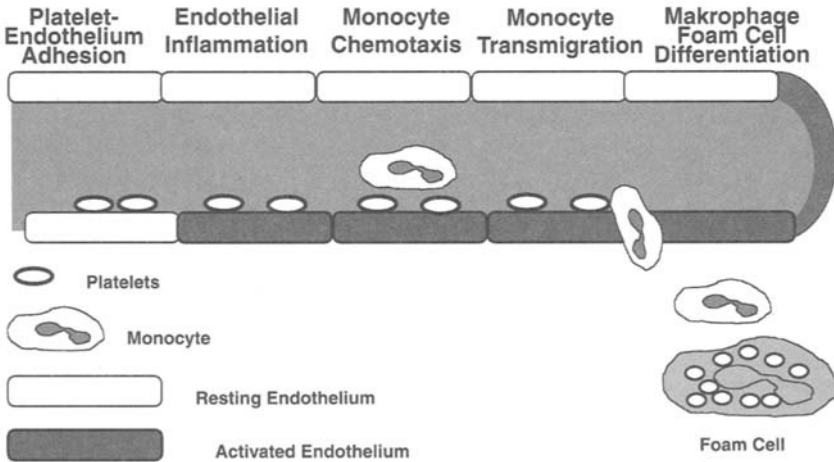
Recently, the occurrence of platelet-derived proatherogenic compounds like PF-4 and NAP-2 in carotid plaques has been documented (136). PF-4 is a potent chemotactic factor for peripheral blood monocytes and has been found to enhance markedly the uptake of oxLDL by macrophages, an important mechanism in the development of unstable atherosclerotic plaques (137). Only recently was it shown that platelet adhesion to the arterial wall is enhanced during atherogenesis (17). Platelet adhesion to endothelium coincides with inflammatory gene expression and precedes atherosclerotic plaque invasion by leukocytes. Prolonged blockade of platelet adhesion in mice deficient in apolipoprotein E profoundly reduced leukocyte accumulation in the arterial intima and attenuated atherosclerotic lesion formation in the carotid artery bifurcation, the aortic sinus, and the coronary arteries (Fig. 8) (17). Moreover, chronic platelet activation accelerates atherosclerosis (18). Thus, platelet-induced inflammation of the vessel wall plays a major role in initiation of the atherogenetic process (Fig. 9).

## CONCLUSIONS

Platelets play a critical role in acute and chronic inflammation. Major advances have been achieved in the understanding of the molecular mechanisms involved in platelet interaction with inflammatory cells. The interaction of platelets with leukocytes and endothelial cells results in substantial activation of these cell types that changes their chemotactic, adhesive and proteolytic properties significantly. Specific cell adhesion receptors such as selectins, immunoglobulin-type receptors, and integrins are involved in regulation of platelet adhesion with inflammatory cells. Thereafter, release of platelet-dependent proinflammatory mediators substantially triggers the inflammatory response in the microenvironment of the site of platelet adhesion. Thus, platelet adhesion and subsequent release reaction are critical mechanisms involved in



**Fig. 8.** Chronic GPIb inhibition of platelet-endothelium adhesion substantially inhibits atheroprogession. Plaque extension in the carotid artery in apolipoprotein E (ApoE)-deficient mice treated or not treated chronically with anti-GPIb monoclonal antibody (17).



**Fig. 9.** Critical role of platelet adhesion in atherogenesis.

platelet-mediated inflammation and might represent targets for future therapeutic strategies in a variety of inflammatory diseases.

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