Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium

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Abstract

Platelet-dependent thrombus formation is a key event in the pathogenesis of acute myocardial infarction (AMI). Platelets mediate both thrombotic occlusion of the entire epicardial coronary artery and also accumulate in the microcirculation resulting in impairment of microcirculation and provoking myocardial ischemia during reperfusion. In the past, our understanding of platelet function in myocardial infarction (MI) and reperfusion has extended substantially resulting in development of novel and clinically effective treatment strategies. This review summarizes the mechanisms of coronary thrombosis and consequences of platelet accumulation in reperfused myocardium. Basic pathophysiological mechanisms of platelet adhesion and secretion are reviewed first, followed by the description of the molecular steps involved in platelet-mediated thrombus formation around an atherosclerotic plaque. Next, the role of platelets in inflammation is summarized with the focus on platelet–endothelium and platelet–leukocyte interactions.

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1. Introduction

Acute myocardial infarction (AMI) is a major public health problem and a leading cause of death in the western world. Platelets play a major role in the pathophysiology of AMI. At site of a vulnerable coronary plaque, platelets attach to the vessel wall and initiate thrombotic occlusion of the coronary vessel leading to myocardial ischemia and infarction. Besides thrombus formation in the epicardial arteries, platelet microembolization and accumulation within the microcirculation of the ischemic myocardium play a major role in microcirculatory arrest thus promoting tissue damage.

Prompt reperfusion of ischemic myocardium is critical for restoring normal heart function. The primary objective of reperfusion therapy in myocardial infarction (MI) has been to restore normal epicardial blood flow in the infarct-related coronary artery. However, this return of blood flow can paradoxically enhance the destruction of reversibly damaged myocytes, thereby leading to the progression of myocardial dysfunction and infarction [1–3]. Moreover, even among patients who achieve normal epicardial flow, the myocardial perfusion may be inadequate, which is characterized as no reflow at the level of the myocardial and coronary microcirculation [4]. Such patients with normal epicardial flow but impaired microvascular perfusion have a high risk for the development of congestive heart failure, arrhythmia and death [5,6].

Understanding the molecular mechanisms of platelet function in reperfusion will improve the treatment options and enhance myocardial salvage in patients with acute coronary syndromes including myocardial infarction. This article reviews the pathophysiological role of platelets in myocardial reperfusion injury, focusing on (a) the molecular mechanisms of thrombus formation and microembolization at sites of atherosclerotic plaques in epicardial coronary arteries and (b) platelet interaction with the endothelium and leukocytes and its consequence for inflammation, microcirculation and therapy.
2. Platelets, coronary thrombosis and myocardial infarction

Myocardial infarction is due to atherosclerotic alterations of the coronary vessel wall and thrombotic occlusion. Critical steps in the development of MI are (a) thrombotic occlusion of an epicardial coronary artery at a disrupted/eroded atherosclerotic plaque, (b) microembolization of atherothrombotic platelet-rich aggregates, (c) platelet-mediated vasoconstriction, (d) enhanced intravascular thrombus formation in the microcirculation and (e) platelet-mediated inflammatory reactions in the ischemic myocardium (Fig. 1). Combination of these events determines the degree of myocardial ischemia and cardiac contractile dysfunction and thereby the clinical presentation of the disease. Platelet-dependent thrombus formation in the macroscopic and microscopic coronary vessels is, thus, a major event in the development of MI. Reperfusion strategies aim to dissolve the thrombotic plug inside the epicardial coronary artery either through administration of fibrinolytic agents or through direct percutaneous coronary intervention (PCI).

Fissure or rupture of the advanced atherosclerotic lesion leads to endothelial denudation and exposure of the thrombogenic subendothelial matrix to circulating platelets and initiates platelet recruitment to the injured vessel wall in a process that closely resembles primary hemostasis (Fig. 2). The subendothelial extracellular matrix is made of a network of collagen fibrils that are embedded in a gel of proteoglycans, glycoproteins and water. Also present is von Willebrand factor (vWF) that is secreted by the endothelium. A smaller portion of vWF is secreted into the abluminal space where it adheres to collagen, while the majority of vWF is released into the plasma compartment [7].

Among the constituents of the subendothelial matrix, fibrillar collagen has been proposed to be of major importance for platelet adhesion and aggregation at sites of vascular injury [7]. Interestingly, with increasing age and advancing atherosclerosis, the composition of vascular wall collagen changes to higher amounts of type I collagen [8]. A current hypothesis supported by numerous in vitro studies suggests that the first contact between circulating blood platelets and the vessel wall lesion (platelet tethering) is established by an interaction of the platelet receptor for von Willebrand factor (GPIb-V-IX) with collagen-immobilized vWF (Fig. 2B) [7]. Exposure of collagen fibrils to the bloodstream following plaque rupture or fissure initiates the association of circulating vWF with exposed collagen and allows the subsequent binding of platelets via GPIb-V-IX. The interaction of vWF with GPIb-V-IX is characterized by a fast association rate that can tether platelets to the exposed subendothelium at high shear rates. This event is favored by the multimeric nature of vWF, providing a high local density of this protein and resulting in formation of multiple bonds.

Recent in vivo evidence suggests that, apart from GPIb-V-IX, another platelet membrane receptor, the glycoprotein VI, is strictly required for the initial platelet tethering following vascular injury. Over the past years, GPVI has been established as the central platelet collagen receptor that is essential for platelet adhesion and aggregation on immobilized collagen in vitro [9]. Unlike GPIb-V-IX, GPVI binds directly to subendothelial collagen and mediates the activation of different adhesive receptors, including integrins αIIbβ3 and αvβ3 [9] required for stable platelet arrest. GPVI is a 60–65 kDa type I transmembrane glycoprotein belonging to the immunoglobulin superfamily that forms a complex with the FcR γ-chain at the cell surface in human and mouse platelets [9]. Recently, we were able to demonstrate that direct GPVI–collagen interactions are crucial for initial platelet tethering and subsequent stable platelet adhesion and aggregation at sites of arterial injury [10]. In a mouse model of vascular injury, inhibition or absence of the major platelet collagen receptor GPVI almost completely abolished platelet tethering following endothelial denudation, as assessed by in vivo fluorescence microscopy and scanning electron microscopy. The strict requirement for GPVI in the process of platelet tethering was confirmed in GPVI-deficient mice where platelets also failed to adhere and aggregate on the damaged vessel wall although expression and function of GPIb-V-IX was not altered under these experimental conditions. These findings establish the critical role of GPVI in the initiation of platelet attachment at sites of vascular injury and unequivocally showed that, in the absence of GPVI, other surface receptors, most importantly GPIb-V-IX, are not sufficient to initiate platelet adhesion and aggregation on the subendothelium in vivo. Thus, it appears that GPIbα and GPVI act in concert to recruit platelets to the subendothelium in vivo (Fig. 2B).

While the physicochemical properties of GPVI–collagen interactions are well defined, the vWF–GPIb-V-IX interactions are known to be characterized by a fast dissociation rate. In other words, GPIbα cannot provide

![Fig. 1. Role of platelets in acute coronary syndrome and reperfusion.](https://academic.oup.com/cardiovascres/article-abstract/61/3/498/404313 by guest on 02 February 2019)
stable arrest of platelets to the subendothelial matrix. Hence, platelets tethered to vWF move constantly in the direction of blood flow. During slow surface translocation, occupation or lateral clustering, GPIbα is thought to transmit signal activation of platelet integrin receptors, such as the fibrinogen receptor αIIbβ3 (fibrinogen receptor) and α2β1 (collagen receptor). Interaction of αIIbβ3 and α2β1 with extracellular matrix proteins stabilizes platelet adhesion (firm adhesion) (C). Subsequently, platelets spread and degranulate and thereby recruit additional platelets to the already adherent ones (D). Platelets form microaggregates via fibrinogen “bridges” between two αIIbβ3 receptors (E). Formation of microparticles around the platelet aggregates catalyses thrombin generation and thus fibrin formation that stabilizes the platelet thrombus (F).

Fig. 2. Platelet-dependent formation of a thrombus at an atherosclerotic plaque. Platelets do not adhere to the intact endothelial monolayer under physiological conditions (A). At site of vascular lesions, extracellular matrix proteins like von Willebrand factor (vWF) and collagen (Col) are exposed to the blood. Via the membrane adhesion receptors GPIbα and GPVI platelets loosely adhere to the subendothelium (B). This initial adhesion results in platelet activation and “opening” of the integrin receptors αIIbβ3 (fibrinogen receptor) and α2β1 (collagen receptor). Interaction of αIIbβ3 with extracellular matrix proteins stabilizes platelet adhesion (firm adhesion) (C). Subsequently, platelets spread and degranulate and thereby recruit additional platelets to the already adherent ones (D). Platelets form microaggregates via fibrinogen “bridges” between two αIIbβ3 receptors (E). Formation of microparticles around the platelet aggregates catalyses thrombin generation and thus fibrin formation that stabilizes the platelet thrombus (F).
ponents (in particular ADP) are released during the adhesion (secretion) that reinforce the adhesion process not only by an “autocrine” mechanism but also by a “paracrine” mechanism, namely by stimulation and recruitment of resting platelets from the circulation and by inducing them to undergo aggregation with already adhering platelets (platelet recruitment). Highly thrombotic material, exposed during plaque rupture through the fibrous cap, promotes further platelet aggregation.

The interaction of circulating platelets with adherent platelets proceeds through activated \( \alpha_{\text{IIb}}\beta_3 \) integrin receptors. During the primary phase (primary aggregation), the platelets are loosely linked to each other by “fibrinogen bridges” [12] (Fig. 2E). In the resting state, soluble plasma fibrinogen cannot bind to the platelet surface as binding sites for fibrinogen in the region of the glycoprotein IIb–IIIa complex only become accessible after activation. The binding of GPIIb–IIIa is strongly dependent on \( \text{Ca}^{2+} \) and leads to the formation of platelet aggregates (Fig. 2E). Importantly, the initial binding of fibrinogen to GPIIb–IIIa is a reversible process that can be followed seconds to minutes later by an irreversible stabilization of the fibrinogen linkage to the GPIIb–IIIa complex. During platelet aggregation, recruited platelets release potent platelet agonists including thrombin, ADP and thromboxane, initiating the incorporation of additional platelets into the growing aggregate [7]. Furthermore, activated platelets shed off their membrane surface small microparticles that are characterized by high procoagulant activity. Microparticles catalyze formation of thrombin around a platelet microaggregate, thereby inducing fibrin generation and consolidation of the coronary thrombus (Fig. 2E).

3. Platelets and microembolization

Atherosclerotic plaque rupture is a key event in the pathogenesis of acute coronary syndromes and during coronary interventions. However, it does not always result in complete thrombotic occlusion of the entire epicardial coronary artery with subsequent acute myocardial infarction. In milder forms, the result can be the embolization of atherothrombotic debris and/or platelet microaggregates in the coronary microcirculation [13,14] (Fig. 3). Embolization of platelet aggregates was originally believed to be an uncommon event, chiefly confined to revascularization of degenerated, aged, venous bypass grafts. The development of the Folts cyclic flow model contributed significantly to our understanding of the role of platelets in microembolization [15]. In patients with acute coronary syndrome, periodic coronary flow reduction occurs often, which leads to transient myocardial ischemia. This is caused by periodic platelet-mediated thrombi developed in the stenosed epicardial coronary artery, which temporarily cut off the coronary blood flow. Then the platelet-mediated thrombus embolizes distally, thereby suddenly restoring coronary blood flow. However, microembolization leads to decreased regional coronary blood flow distal to the stenosis, to transient ischemia and to unstable angina or non-ST-segment elevation myocardial infarction. Because of newer diagnostic imaging modalities that include magnetic resonance and myocardial contrast echocardiography, and due to the development of technical devices that trap thrombembolic material, microembolization and microvascular obstruction has been documented in a far greater proportion in patients than ever perceived. New evidence has underscored the frequency and prognostic importance of atherosclerotic embolization in the microvasculature. Using myocardial contrast echocardiography, Ito [4] demonstrated that more than 25% of patients with successful revascularization following acute myocardial infarction and normal epicardial flow (as assessed by coronary angiography) did not have adequate tissue reperfusion. Applying more sensitive markers of myocardial necrosis, such as troponin, the incidence of thrombotic embolization is even higher [16]. Accordingly, peripheral platelet microaggregates in the supply region of the coronary arteries can be observed in most patients with unstable angina and myocardial infarction [17].

Microembolization and microvascular obstruction is linked to an unfavorable long-term clinical prognosis [16]. Apart from mechanical thrombotic obstruction, platelets in embolized vessel segments induce temporary vasospasms through the release of serotonin, thromboxane A2 and free radicals. Thus, platelets are involved both in microvascular obstruction by thrombotic emboli and temporary vasospasms that results in inadequate perfusion and prolonged tissue ischemia of the affected myocardial regions despite successful revascularization and normal epicardial blood flow. This can lead to minor myocardial injury (“infarctlets”) or extended myocardial necrosis with elevation of creatin kinase, regional myocardial contractile dysfunction and potentially arrhythmias (Fig. 3). Microembolized myocardium is further characterized by perfusion-contraction mismatch with reduced contractile function and unchanged blood flow [14].
4. Platelets and microcirculation

Nowadays the thrombotic vascular occlusion can be reopened and reperfusion is achieved by the rapid use of fibrinolytic agents or direct angioplasty in case of acute myocardial infarction. The revascularization of the coronary artery is a prerequisite for the reestablishment of an adequate metabolic supply of the afflicted myocardial area. However, apart from rapid and successful reperfusion of the epicardial macrovessel, the regeneration of the ischemic myocardial area largely depends on the integrity and recovery of the microcirculation in the vicinity of the postischemic myocardium. Myocardial ischemia reperfusion exhibits all the characteristics of an acute inflammatory response including cytokine secretion, expression of cell adhesion molecules, neutrophil infiltration and increased microvascular permeability [18]. In the past, the critical role of platelets as “inflammatory cells” has been recognized because activated platelets adhere to the endothelium and to leukocytes and induce inflammatory reactions through a release of proinflammatory compounds.

4.1. Platelet secretion and inflammation

In recent years, increasing evidence has indicated that inflammatory mechanisms play a pivotal role in the pathophysiology of reperfusion. Besides their role in thrombosis, platelets play a critical role in inflammation [19]. Chemotaxis is a central mechanism to recruit inflammatory cells to a site of tissue damage or repair. 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 synthetized after stimulation [20]. Moreover, platelets may by direct cell contact modulate the chemotactic properties of other cells including leukocytes [21,22] and endothelium [23,24]. Platelets contain three different forms of storage granules: dense bodies or dense granules, α-granules and lysosomes. The granules are characteristic for platelets and serve as storage sites for proteins and other low molecular weight compounds (Table 1).

4.1.1. Dense granules

The dense granula 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 [25]. Stimulation of monocytes through serotonin induces secretion of lymphocyte chemoattractant activity (IL-16) that may promote the recruitment of T lymphocytes into an inflammatory focus [26]. Other dense granule constituents (histamin, ATP) have been shown to support the proinflammatory reaction in the microenvironment of platelet adhesion [27].

4.1.2. Alpha-granules

Alpha-granules represent the major granule population in size and number [28]. The α-granules are typical secretory vesicles which 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. Two specific platelet proteins, β-thromboglobulin (β-TG) and platelet factor-4 (PF-4) are localized in α-granules together with proteoglycans. The latter include a family of β-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 β-TG and PF-4. Purified PF-4 lacks chemotactic activity for leukocytes but costimulation of leukocytes with TNF-α results in exocytosis of secondary granule markers or tight adhesion to different surface proteins [29]. PF-4 not only affects neutrophils, but it also induces the release of histamine by basophils and plays a role in eosinophil adhesion [29]. β-TG represents a chemokine for neutrophils, monocytes and lymphocytes. Epithelial
neutrophil-activating protein 78 (ENA-78) is another α-granule constituent that stimulates neutrophil activation [30]. Among the mitogenic factors present in platelet α-granules, the specific platelet-derived growth factor (PDGF) is present together with transforming growth factor-β (TGF-β), endothelial cell growth factor (ECGF), epidermic growth factor (EGF), vascular endothelial growth factor (VEGF), vascular permeability factor (VPF), basic fibroblast growth factor (bFGF) and insulin-like growth factor-I (IGF-I) [28]. PDGF and TGF-β are growth factors, which exert chemotactic properties on smooth muscle cells, macrophages, monocytes and fibroblasts [28]. Inhibition of PDGF by neutralizing anti-PDGF antibodies inhibits platelet-induced migration of smooth muscle cells [28,31]. Recently, platelet-derived PDGF and TGF-β have been found to induce expression of VEGF in smooth muscle cells [32], which is an endothelial mitogen and chemokine that stimulates endothelial cell migration. The chemokines RANTES and macrophage inflammatory protein-1α (MIP-1α) belong to the CC chemokines and are potent chemokines for basophils, eosinophils, T lymphocytes and monocytes [28].

4.1.3. 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 [20].

4.1.4. Cytosol

Other important proinflammatory mediators (interleukin-1β [IL-1β], CD40 ligand [CD40L]) are present in the cytosol and are generated from mRNA [33] and are release upon activation [34]. IL-1β is a central mediator in the cytokine cascade and a potent activator of vascular cytokine production. CD40L is structurally related to TNF-α and translocates within seconds of activation to the platelet surface. CD40L stimulates endothelium to express ICAM-1, VCAM-1 and E-selectin, thus modulating leukocyte–endothelium interaction. Moreover, CD40L stimulates platelet secretion [35] and stabilizes platelet aggregation by interference with GPIIb–IIIa [36].

4.1.5. Lipid mediators

In addition to granule- and cytoplasma-stored substances, platelets release lipid mediators upon activation including platelet-activating factor (PAF), thromboxane A2 (TxA2), prostaglandin E2 (PGE2), platelet-derived lysosphatidic acid (LPA) and sphingosine-1-phosphate (SP-1) [37]. PAF is a phosphoglyceride generated from the phospholipids of cell membranes. PAF promotes leukocyte–endothelium interaction and favors diapedesis of leukocytes [38]. TxA2 is another potent platelet mediator derived from the platelet membrane. Upon platelet activation, TxA2 is synthesized de novo from AA through the action of platelet cyclooxygenase-1 (COX-1), an enzyme that is inhibited irreversibly by aspirin. TxA2 is known to induce platelet aggregation at sites of platelet activation. In addition, the AA-metabolite acts as a potent proinflammatory mediator, inducing leukocyte adhesion to and migration across activated endothelial cells [39]. Another rich sources of proinflammatory AA-metabolites are platelet microparticles (MP), membrane blebs that are shed from the surface of activated platelets by membrane vesiculation. Platelet microparticles have been shown to increase the adhesion of monocytes to endothelial cells in a time- and dose-dependent manner [40]. In line with this finding, MP-derived AA 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 [37]. Moreover, LPA leads to the activation of NF-κB 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 [37]. Hence, LPA released during platelet–endothelial cell interaction at sites of endothelial dysfunction could function in a paracrine manner directly modulating inflammatory and proliferative responses in the vascular wall.

4.2. Platelet–endothelium interaction

In the past, numerous studies have shown that platelets can adhere to the intact endothelial monolayer and to substantially modulate endothelial cell function [41–43]. Thus, under certain pathophysiological circumstances, endothelial denudation and exposure of subendothelial matrix are not required for platelet adhesion to the vascular wall. Adherent platelets release a variety of pro-inflammatory 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, which is thought to play a key role in acute and chronic inflammation. Normal “resting” endothelium represents a non-adhesive and non-thrombogenic surface that prevents extravasation of circulating blood cells. In contrast, activated endothelial cells are pro-adhesive 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 towards the vessel wall even under high shear stress. Similar to the recruitment of leukocytes [44], 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. 4). Whereas the adhesion receptors involved in platelet attachment to the subendothelial matrix, e.g. following rupture of an athero-
sclerotic plaque, have been well defined during the past decade, few studies have focused on the molecular determinants that promote the interaction between platelets and the intact vascular endothelium.

4.2.1. P-selectin

The initial loose contact between circulating platelets and vascular endothelium (“platelet rolling”) is mediated by selectins, present on both endothelial cells and platelets [45–47]. 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. In addition, P-selectin is stored in platelet α-granules and can rapidly translocate on the platelet surface upon activation. Endothelial P-selectin has been demonstrated to mediate platelet rolling in both arterioles and venules in acute inflammatory processes, such as ischemia/reperfusion [48–50] (Fig. 4). E-selectin, which is also expressed on inflamed endothelial cells, allows a loose contact between platelets and endothelium in vivo, too [51]. 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 [46,47].

So far, few studies have addressed the exact nature of the ligands expressed on platelets that binds 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 [52,53]. However, unlike the interaction with the selectins on leukocytes, the interaction with platelets does not appear to involve the P-selectin glycoprotein ligand-1, since the presence of this mucin-like glycoprotein on the platelet membrane has not yet been documented and platelet rolling is not inhibited by an anti-PSGL-1 monoclonal antibody in vivo (personal communication with Dr. Massberg).

4.2.2. Glycoprotein Ib-IX-V

Another sialomucin that has been identified as a potential counterreceptor for platelet P-selectin is the leucine-rich glycoprotein Ib-IX-V, the vWF receptor complex. Romo et al. [54] have demonstrated recently that cells expressing P-selectin roll on immobilized GPIba. Platelet rolling on activated endothelium can be inhibited by antibodies against both P-selectin and GPIba, indicating that the vWF receptor mediates both platelet adhesion to the subendothelial matrix and to intact endothelial cells. Future studies will have to clarify if the association of GPIba with P-selectin leads to platelet activation similar to GPIba–vWF interaction.

Interactions of selectins with their counterreceptors are characterized by high on- and off-rates, enabling platelets to rapidly attach to the endothelial monolayer with high resistance to tensile stress, explaining why adherent platelets can oppose the drag created by the shear rates present particular 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 tailing edge of platelets, where the tension is greatest resulting in forward rotational movement (rolling) from torque imposed by the blood flow. However, due to their biophysical characteristics, selectin–ligand interactions are not sufficient to promote firm adhesion of platelets in the bloodstream. This implicates 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.
4.2.3. Integrins

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 (αIIbβ3) is the major integrin on platelets and plays a key role in platelet accumulation on activated endothelium. In the presence of soluble fibrinogen, αIIbβ3 mediates heterotypic cell adhesion to αvβ3-expressing cells including endothelial cells [41,55]. Moreover, platelets firmly adhere to activated endothelial cells via αIIbβ3, a mechanism that can be blocked by antagonists of β3-integrins [41]. In vivo, firm platelet adhesion to the endothelium can be inhibited by anti-αIIbβ3 mAb and platelets defective in αIIbβ3 do not firmly adhere to activated endothelial cells [41]. Taken together, this indicates that besides mediating platelet aggregation the platelet fibrinogen receptor is of paramount importance in mediating firm attachment of platelets to the vascular endothelium (Fig. 5).

Only few integrins have been reported to be expressed on the luminal side of endothelial cells. Among those, the vitronectin receptor (αvβ3) 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-1β or thrombin [56]. Inhibition of αvβ3 attenuates platelet–endothelial cell interaction [41]. Hence, both platelet αIIbβ3 and endothelial αvβ3 are involved in mediating firm adhesion of platelets to the luminal aspect of activated endothelial cells. However, direct binding of αIIbβ3 to endothelial αvβ3 has not been reported so far.

In fact, heterotypic cell adhesion through αIIbβ3 and αvβ3 requires the presence of fibrinogen, which bridges the platelet fibrinogen receptor to the endothelial vitronectin receptor [55]. 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. 5) [42,57]. The affinity of the platelet αIIbβ3 for its ligand underlies strict regulation and increases with platelet activation (“inside out-integrin signaling”, see above). Although even non-activated αIIbβ3 can bind to immobilized fibrinogen, platelet activation during the initial contact...
between platelets and endothelial cells ("platelet rolling"), e.g., via GPIbα-P-selectin interaction, might enhance the fibrinogen binding capacity of αIbβ3 and thereby facilitates subsequent firm platelet adhesion.

4.2.4. Adhesion receptors of the immunoglobulin type

Another adhesion molecule that contains a fibrinogen recognition site and acts as an endothelial fibrinogen receptor, promoting fibrinogen deposition at the inflamed endothelium, is the intercellular adhesion molecule (ICAM)-1. ICAM-1-fibrinogen interactions have been demonstrated to participate in the process of firm attachment of leukocytes to inflamed endothelial cells in vivo [44]. Likewise, an ICAM-1-dependent bridging mechanism exists in the accumulation of platelets both in vitro and in vivo [42,50]. Correspondingly, platelet adhesion to activated endothelial cells is largely reduced in mice lacking ICAM-1, indicating that both ICAM-1 and the vitronectin receptor participate in the recruitment of platelets to the inflamed vasculature. Recently, two members of the JAM adhesion receptor family have been identified in platelets, JAM-1 [58] and JAM-3 [59]. Platelets constitutively express JAM-1 (F11R) and inhibiting JAM-1 by blocking mAb reduced platelet adhesion to TNFα-treated endothelial cells [58] (Fig. 5).

In summary, platelet–endothelial cell interactions are a multistep process, in which selectins or 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. 5).

4.3. 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. 6). 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. The IL-1β activity expressed by platelets appears to be associated with the platelet surface [60] and co-incubation 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-1β-dependent manner [61,62].

However, platelet IL-1β does not only modify endothelial release of chemotactic proteins, it also has the potential to increase endothelial expression of adhesion molecules. Surface expression of ICAM-1 and αIβ3 on endothelial cells is significantly enhanced by activated platelets via IL-1β [56,62]. Both enhanced chemokine release and upregulation of endothelial adhesion molecules through platelet-derived IL-1β act in concert and promote neutrophil and monocyte adhesion to the endothelium. IL-1β-dependent expression of early inflammatory genes, such as MCP-1 or ICAM-1, involves the activation of the transcription factor nuclear factor kappa B (NF-κB). Transient adhesion of platelets to the endothelium initiates degradation of IκB and supports activation of NF-κB in endothelial cells, thereby inducing NF-κB-dependent chemokine gene transcription [61]. Parallel to this finding, transfection of “decoy” κB oligonucleotides or a dominant negative IKK mutant attenuates platelet-induced nuclear translocation of NF-κB and MCP-1 secretion in endothelial cells [62]. Likewise, platelet-induced NF-κB-activation was largely reduced by IL-1β antagonists, supporting the notion that platelet IL-1β is the molecular determinant of platelet-dependent activation of the transcription factor. Taken together, platelet-derived IL-1β initiates NF-κB-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 RANTES, which has been identified to trigger monocyte arrest on inflamed and atherosclerotic endothelium [63]. Deposition of platelet RANTES induces monocyte recruitment mediated by P-selectin. 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-α and Fas ligand. CD40L was originally thought to be restricted to CD4+ T-lymphocytes, mast cells and basophils. Henn et al. [64] showed that platelets store CD40L in high amounts and release CD40L within seconds following activation in vitro and in vivo. 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. In addition, platelet CD40L enhanced the expression of endothelial

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**Figure 6. Platelet-induced endothelial inflammation.**

[Diagram of platelet-induced endothelial inflammation with labels for Adhesion, Chemotaxis, Proteolysis, Migration, and Nucleus.]
adhesion receptors including E-selectin, VCAM-1 and ICAM-1, all molecules that mediate the attachment of neutrophils, monocytes and lymphocytes to the inflamed vessel wall. Hence, like IL-1β, 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 initiate the expression and release of matrix degrading enzymes, the matrix metalloproteinases (MMPs). These enzymes, which degrade extracellular matrix proteins, significantly contribute to destruction and remodeling of inflamed tissue. Adhesion of activated platelets to endothelial cells results in generation and secretion of MMP-9 and of the protease receptor uPAR on cultured endothelium [35]. The endothelial release of MMP-9 was dependent both on 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 resulted in substantial release of CD40L in the absence of any further platelet agonist (Fig. 7). These results propose that the release of platelet-derived proinflammatory mediators like CD40L is dependent on GPIIb–IIIa-mediated adhesion. This mechanism may be pathophysiologically important to localize platelet-induced inflammation of the endothelium at a site of platelet–endothelium adhesion.

4.4. Platelet–leukocyte interaction

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 towards the site of platelet accumulation. Similar to platelet adhesion to the vessel wall, leukocyte recruitment to vascular endothelium requires multistep adhesive and signaling events including selectin-mediated rolling, leukocyte activation and integrin-mediated firm adhesion and diapedesis [44] (Fig. 8). On leukocytes, members of the β2-integrin family, LFA-1, MAC-1 and p150.95 as well as β1-integrins interact with endothelial counterligands such as ICAM-1, surface-associated fibrinogen [44] or vascular cell adhesion molecule-1 (VCAM-1) to mediate the described heterotypic cell interaction. At sites of platelet adhesion to endothelium or subendothelium, leukocyte infiltration can occur through interactions with platelets and fibrin [65,66]. Similar to the leukocyte–endothelium adhesion a sequential adhesion process of leukocytes to adherent platelets has been proposed. Leukocyte adhesion to platelets involves surface expression of P-selectin on activated platelets and binding to PSGL-1, the counterreceptor present on neutrophils and monocytes [67]. Diaco et al. [68,69] have demonstrated earlier that leukocytes tether, roll and subsequently rest on activated platelet monolayers via sequential action of platelet P-selectin and ICAM-2 binding to their leukocyte counterreceptors PSGL-1 and CD11b/CD18, respectively (Fig. 8). This suggests that platelets attached to the vessel wall may recruit leukocytes. In addition, P-selectin/PSGL-1-dependent platelet–leukocytes interaction brings platelets into close vicinity with neutrophils and may facilitate leukocyte activation by platelet proinflammatory mediators. Recent data indicated that GPIbα and JAM-3 on platelets are potential counterreceptors for MAC-1 [59] and that they mediate mechanism of platelet–leukocyte adhesion. Furthermore, ICAM-2 and αιββ3-associated fibrinogen have also been proposed to mediate MAC-1-dependent platelet–leukocyte adhesion. The in vivo relevance of these mechanisms has yet to be elucidated.

Ligation of PSGL-1 by P-selectin enhances phosphorylation of tyrosine residues and activates MAP-kinases [70] in leukocytes that in turn activate β2-integrin mediated cell attachment to ICAM-1 [71] (Fig. 9). Furthermore, activated platelets induce the expression of cytokines/chemokines (such as IL-1β, MCP-1 and IL-8) as well as tissue factor by quiescent monocytes [72]. This process was dependent on P-selectin binding to its counterreceptor present on monocytes. Zimmerman et al. [73] demonstrated that
PAF derived from platelets acts as a juxtacrine signal that alters the activity of β2 integrins on myeloid leukocytes and works in concert with P-selectin at the surfaces of endothelial cells [73]. Thus, platelets either immobilized on a surface or activated in suspension express a complete machinery to recruit leukocytes: (a) platelet P-selectin is a mediator of the first contact (tethering), (b) interaction of platelet P-selectin with its counterreceptor PSGL-1 on leukocytes induces signaling events relevant for MAC-1 activation and (c) the activated β2-integrin (MAC-1) on leukocytes allows and reinforces firm platelet–leukocyte adhesion through binding to counterreceptors (ICAM-2, fibrinogen bound to GPIIb–IIIa, GPIbα, JAM-3) present on the platelet surface (Fig. 8).

5. Consequences for therapy

Among patients with successful recanalization of the infarct vessel, myocardial malperfusion occurs in 22 to 50% despite an open epicardial artery. Microvascular malperfusion in patients with MI detected by magnetic resonance imaging is associated with a poor prognosis [74], which implies the need for improving the pharmacological therapy during reperfusion. In the past, numerous studies in animals have demonstrated that platelets contribute to reperfusion injury by promoting inflammatory reactions within the ischemic myocardium [75–77]. On the other hand, it has been shown in animals that platelets may also reduce reperfusion injury by a number of mechanisms, such as release of nitric oxide or antioxidants that result in attenuation of platelet activation, which may lead to cardioprotection [78]. Moreover, aggregating platelets release TGF-β1, TGF-β3 has been described to have a cardioprotective activity against myocardial ischemia–reperfusion injury [79,80]. However, the clinical relevance of these experimental observations still has to be validated.

Limited data on the role of platelets for reperfusion injury is available in humans. To date, several anti-inflammatory and anti-thrombotic strategies have been evaluated in humans to improve microcirculation and myocardial salvage in patients with MI [81]. A strategy to limit reperfusion injury uses the important role of membrane-bound adhesion molecules that attach platelets to leukocytes and to the vascular endothelium. Monoclonal antibodies against specific adhesion receptors effectively eliminate the function of the corresponding receptor. The most investigated receptors are P-selectin, present on platelets and the endothelium, CD11/CD18, present on leukocytes, and the fibrinogen receptor GPIIb–IIIa on platelets. Numerous animal studies have strongly supported the use of receptor-blocking antibodies as adjunctive reperfusion therapy. However, recent human trials have yielded disappointing results [81]. The most promising advance in the treatment of reperfusion is directed against the platelet-mediated microcirculatory disturbance in the area at risk of the ischemic myocardium. Neumann et al. [82] and Ott et al. [83] have found that platelet–leukocyte binding is increased in patients with acute myocardial infarction as well as in patients with unstable angina. Moreover, others have shown that platelets and neutrophils interact in regulating the vascular tone in arteries injured by angioplasty and that platelets and neutrophils act synergistically in provoking postreperfusion cardiac dysfunction [76]. In a murine stroke model of ischemia/reperfusion, inhibition of platelet GPIIb–IIIa led to a decrease in platelet accumulation in the ipsilateral hemisphere [77]. Furthermore, in patients with acute myocardial infarction, platelet–leukocyte interactions modulated Mac-1 expression on monocytes and glycoprotein Ibα–IIIa blockade interferes substantially with this mechanism [82]. Consequently, Neumann et al. studied the effect of GPIIb–IIIa antagonism in 200 patients with acute MI treated by direct PCI. The patients were randomised to receive abciximab or conventional therapy following successful recanalization of the infarct-related coronary artery through PCI [84]. The study showed a substantial improvement in myocardial perfusion and recovery of the left ventricular function in patients receiving the GPIIb–IIIa receptor antagonist abciximab. Thus, antagonism of platelet GPIIb–IIIa improved the recovery of microvascular perfusion and concomitantly enhanced the recovery of contractile function of ischemic myocardium. Other antiplatelet strategies involving aspirin and clopidogrel may also be beneficial in patients with MI but the beneficial effect on reperfusion has to be determined.

The success of the platelet GPIIb–IIIa inhibitors in the treatment of acute MI and as an adjuvant therapy in PCI and intracoronary stenting has led to their consideration as potential agents to enhance the efficacy of fibrinolytic therapy [85]. The fact that thrombin exposed during fibrinolysis is one of the most potent activators of platelet aggregation further supports the utilization of GPIIb–IIIa antagonists in the development of new pharmacologic treatment strategies aimed at increasing patency rates of the infarct related artery without significantly increased
risk of bleeding complications. Despite promising pilot studies, reduced-dose fibrinolytic therapy combined with GPⅡb–Ⅲa therapy [85] provides only marginal improved clinical outcomes with the cost of enhanced bleeding complications.

6. Conclusions

Platelets play a critical role in the pathophysiology of reperfusion. During the last decade, the underlying molecular mechanisms of platelets and reperfusion have been substantially evaluated and new and effective treatment strategies aiming to interfere with platelet interaction with leukocytes and the endothelium, i.e., platelet-mediated inflammation, have been successfully elaborated. New anti-platelet strategies directed against the platelet secretion process and the collagen receptor GPVI might further improve reperfusion.

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References


