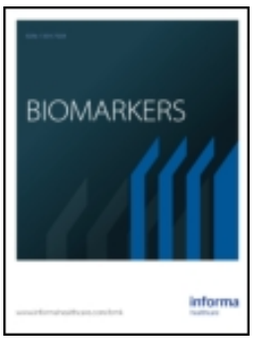

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REVIEW ARTICLE

Impact of platelet phenotype on myocardial infarction

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Abstract

In acute myocardial infarction patients the injured vascular wall triggers thrombus formation in the damage site. Fibrin fibers and blood cellular elements are the major components of thrombus formed in acute occlusion of coronary arteries. It has been established that the initial thrombus is primarily composed of activated platelets rapidly stabilized by fibrin fibers. This review highlights the role of platelet membrane phenotype in pathophysiology of myocardial infarction. Here, we regard platelet phenotype as quantitative and qualitative parameters of the plasma membrane outer surface, which are crucial for platelet participation in blood coagulation, development of local inflammation and tissue repair.

Keywords

Acute coronary syndrome, cardiovascular risk, myocardial infarction, platelet phenotype, platelets

History

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Introduction

Platelets are circulating, small-sized (2-4 μm in diameter) cell fragments which are the central players in the processes of hemostasis and thrombosis. They are formed in the bone marrow by fragmentation of precursor cells (megakaryocytes) (Italiano & Shivdasani, 2003). One megakaryocyte gives rise to about 10 000 platelets. The lifespan of circulating platelets is five to nine days. They are destroyed by the reticuloendothelial cells of the spleen and liver.

In vascular wall injury, e.g. endothelial denudation resulting from intravascular intervention or unstable atherosclerotic plaque rupture, platelets are recruited from the circulation to the subendothelial matrix forming a hemostatic plug. Platelet immobilization in the vascular injury is determined by the specific platelet-vascular wall (adhesion) and platelet-platelet (aggregation) interactions (Figure 1). Platelets are cellular elements that among other peripheral blood components are the first to interact with injured vascular wall. Platelet adhesion is mediated by glycoprotein (GP) Ib-IX-V receptor-complex and collagen receptors, GP VI and alpha(2)beta(1) integrin, located on the platelet surface, and vascular wall collagen and von Willebrand factor (vWF) (Clemetson & Clemetson, 2013). Adhesion to the subendothelial extracellular matrix has been regarded as a trigger of platelet activation, which ultimately leads to the conformational change of integrins expressed on cellular surface (Figure 2). Activated platelets form sites for subsequent accumulation of circulating platelets and leukocytes in the injured vessel wall (Massberg et al., 2004). Release or production of soluble agonists (ADP, thrombin, TxA(2), platelet-activating factor, serotonin, etc.) enhances platelet activation and promotes further accumulation of cells in the injury. Activated platelets not only participate in thrombus formation, but also initiate and/or accelerate inflammatory processes in the vascular wall (Lindemann et al., 2007).

Intact platelets do not show procoagulant phospholipids on their surface. These phospholipids are located on the inner plasma membrane surface and become available by a mechanism specific for activated platelets. Hemker et al. (1983) call this process membrane flip-flop (Figure 3). Membrane flip-flop translocates procoagulant phospholipids (mainly phosphatidylserine) in the intact platelet from inside to outside in the activated platelet. Thus, the intact platelet becomes procoagulant. Clotting factors bind to the procoagulant lipids to form factor X converting enzyme and prothrombinase.

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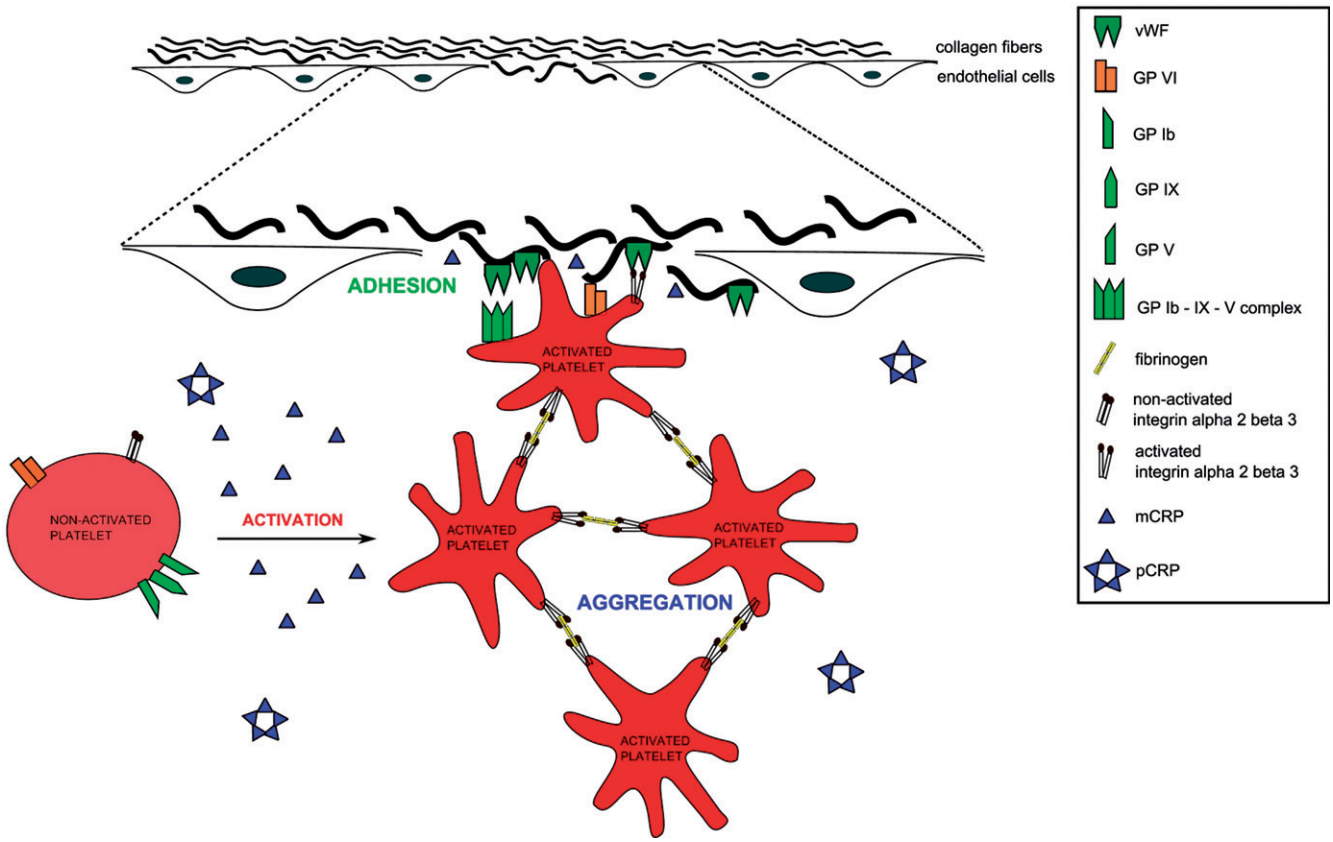

Figure 1. Platelet function in thrombosis. Platelets possess the unique ability to interact with the subendothelial matrix components in areas of injured vascular wall showed in the upper part. Below, the magnification of the process. Specifically, upon activation, platelets display a number of surface receptors that enable them to adhere to collagen fibers and von Willebrand factor, other than to recruit further platelets mainly through the integrin α2β3-fibrinogen cognate interaction and the participation of the mCRP isoform (for comments see the text). In the box on the upper right side, the symbol explanation is given. mCRP: monomeric isoform C-reactive protein; pCRP: pentameric isoform C-reactive protein; GP: glycoprotein; vWF: von Willebrand factor.

Over the past years, it has become clear that platelets are important not only in thrombosis, but also in inflammation. It is widely established that platelets are specialized cells of innate immune defence, modulators of the inflammatory response, and are involved in wound healing. Beyond the obvious functions in hemostasis and thrombosis, platelets are considered to be essential in atherosclerosis, allergy, rheumatoid arthritis and cancer. Moreover, platelets are known to function in innate host defence through the release of antimicrobial peptides and the expression of pattern recognition receptors (Gawaz et al., 2005; Gupalo et al., 2013; Labelle et al., 2011; Lievens & Hundelshausen, 2011).

In atherosclerosis, platelets facilitate the recruitment of inflammatory cells towards the lesion sites. They do so by interacting with circulating leukocytes (lymphocytes, monocytes, neutrophils) and progenitor cells (Figure 4). This cross-talk enforces leukocyte adhesion, activation and transmigration. Formation and emergence of platelet-leukocyte aggregates in the circulation are a manifestation of enhanced cell-to-cell interactions (Satoh et al., 2004). Emergence of platelet-leukocyte aggregates facilitates the development of acute inflammation via stimulation of rolling and subsequent recruitment of leukocytes into the vascular wall. By expressing surface cell adhesion proteins (predominantly, P-selectin) platelets support tethering and rolling of leukocytes in the lesion site. This interaction reduces the flow rate of leukocytes and ensures their firm adhesion with subsequent migration into the vascular wall (Bernardo et al., 2005). High blood level of osteonectin-positive nucleated cells with CD41 positivity could be regarded as an independent indicator of stenosing atherosclerotic lesions (Gabbasov et al., 2007). High content of platelet-leukocyte complexes in the peripheral blood of patients with coronary heart disease is an important component of systemic inflammation. Formation of these aggregates is one of the pathways by which platelets induce inflammation in the vascular wall, which may contribute to the development of atherosclerotic lesions and atherothrombosis (Nijm et al., 2005). In addition, in acute coronary syndromes platelet-leukocyte aggregates appear in the bloodstream much earlier than changes in the standard parameters of platelet activation/activity or in routine markers of myocardial necrosis, such as CK-MB or troponin (Furman et al., 2001).

Platelet ability to regulate the homing of precursor cells in a tissue damage site represents another aspect of platelet involvement in restoration of injured vascular wall. It was widely believed that endothelial and smooth muscle cells of a forming vascular wall, including neointima, arise from adjacent cells that migrate and proliferate into the lesion site. However, it was later demonstrated that bone marrow-derived progenitor cells participate in remodeling and restoration of injured vascular wall (Jiang et al., 2004). Targeted migration of progenitor stem cells into damaged tissue is an important stage in the organism's reaction to damage.

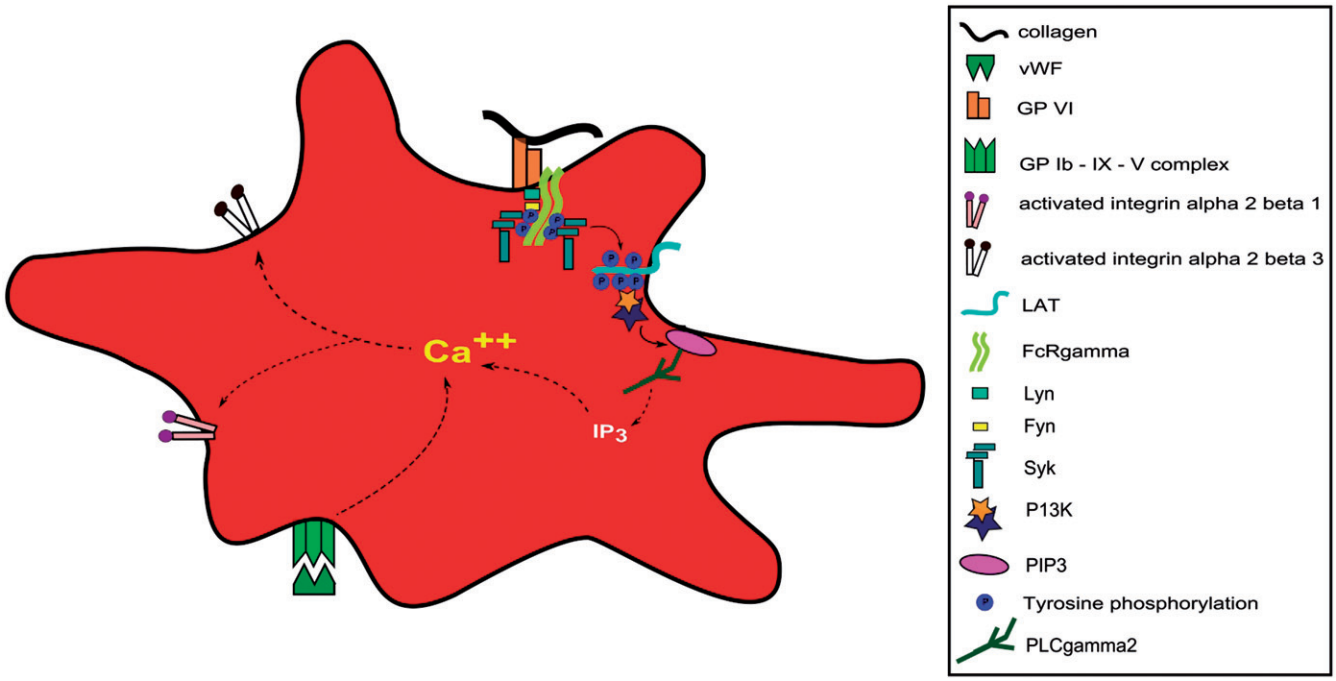

Figure 2. Signal transduction mechanisms upon platelet activation. The binding of GPVI with extracellular collagen fibrils results in clustering and tyrosine phosphorylation of the FcRg by the Src-family kinases Lyn and Fyn. This leads to activation of the tyrosine kinase Syk through its phosphorylation which, in turn, causes the tyrosine phosphorylation also of LAT. This latter triggers the assembly of signalling proteins, such as PI3K, thus leading to the generation of PIP3, and activation of PLCgamma2. As consequence, the IP3 is produced with the end result of mobilization of calcium from intracellular stores and activation of the integrins expressed on cellular surface. Also the binding of vWF to the GPIb-V-IX complex finally results in an intracellular calcium overload leading to the upregulation of integrin a2 affinity. However, this specific signalling pathway has not been completely understood (Zernecke et al., 2005). FcRg: Fc receptor gamma-chain; GP: glycoprotein; IP: inositol (1,4,5)-trisphosphate; LAT: transmembrane adaptor protein; PIP3: phosphatidylinositol (3,4,5)-trisphosphate; PI3K: Phosphoinositide 3-kinase; PLC: phospholipase Cg2; vWF: von Willebrand factor.

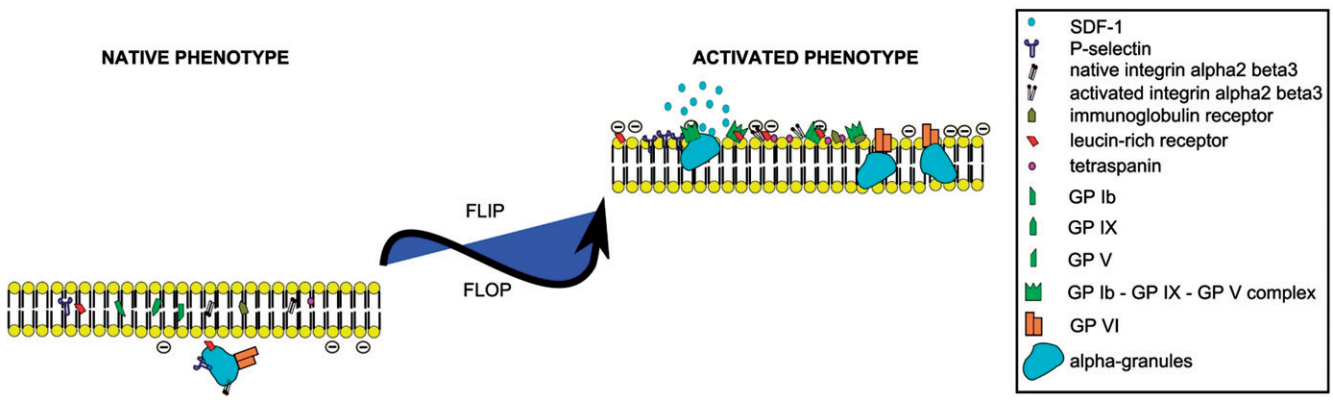

Figure 3. Membrane flip-flop mechanism. Platelet activation allows the translocation of molecules located in the inner surface of the double layer cellular membrane to the outer surface, together with the negatively charged phospholipids. Moreover, the α-granules become incorporated in the plasma membrane, thus allowing both membrane-bound proteins to be expressed on the platelet surface and soluble proteins to be released into the extracellular space (for comments see the text). In the box on the upper right side, the symbol explanation is given. GP: glycoprotein; SDF: Stromal cell-derived factor.

The ability of stem cells to migrate into the ''home'' organ or injury site is determined by specific biochemical signals sent from the ''necessary'' area (Lapidot & Petit, 2002). Stromal cell-derived factor-1 (SDF-1) is produced by bone marrow stromal cells and ''retains'' them in their stem niche. Activated platelets express and secrete SDF-1, which creates the initial gradient of this chemokine in the lesion site and is a major mechanism underlying homing of bone marrow-derived progenitors at lesion sites, e.g. formation of a mural thrombus (Massberg et al., 2006). Thus, platelets actively contribute to formation of niches for homing with subsequent targeted differentiation of progenitor stem cells. Platelets not only participate in thrombotic reaction and inflammatory processes in the vascular wall, but they are also involved in its regeneration (Daub et al., 2006; Langer et al., 2006; Stellos et al., 2009). Jankowski et al. (2001) described the growth-stimulating effect of diadenosine diphosphate (Ap_2A), adenosine guanosine diphosphate (Ap_2G) and diguanosine diphosphate (Gp_2G) in releasable granules of human platelets on cultured vascular smooth muscle cells.

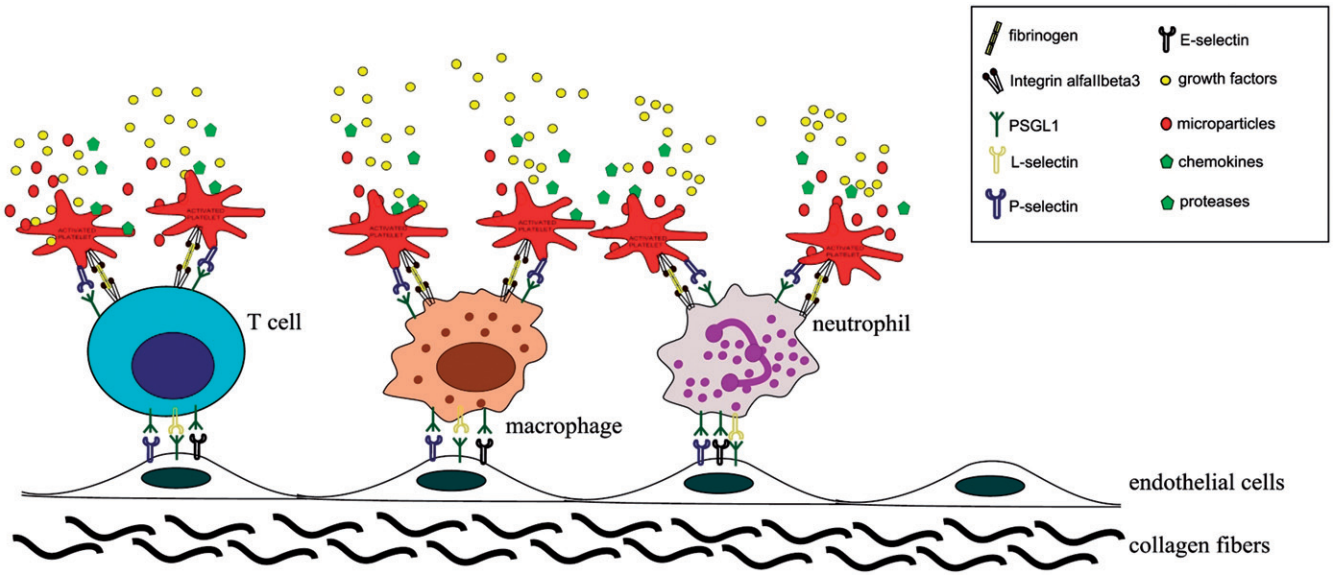

Figure 4. Platelet function in inflammation. Platelets have been shown to take part in both innate and adaptive immune responses by facilitating the recruitment of inflammatory cells, including T cells, monocytes and neutrophils, towards the lesion sites. They do so by expressing surface cell adhesion proteins (predominantly, P-selectin and integrin αIIβ3) that address the leukocyte homing and incorporation into atherosclerotic plaques. Moreover, platelet action also depends on the production of soluble factors, such as growth factors, chemokines, proteases and by releasing microparticles (for comments see the text). In the box on the upper right side, the symbol explanation is given. PSGL: P-selectin glycoprotein ligand.

Platelet phenotype

Here we regard platelet phenotype as quantitative and qualitative parameters of the plasma membrane surface (membrane phenotype), which are crucial for platelet participation in blood coagulation, immune reactions and tissue repair after damage. The modification variability of platelet membrane phenotype, which is determined by deviations from native (non-activated) forms (Figure 3), has some specific features. In contrast to the majority of other cell types, nucleus-free platelets cannot adapt to environmental changes by producing their own proteins. However, there is evidence of residual protein synthesis via megakaryocytic mRNA (Denis et al., 2005). With preserved parental mRNA, platelets possess the translational machinery needed for protein synthesis. Protein production varies in response to activation, and the proteins are capable of modifying the phenotype and functional activity of platelets (Thon & Devine, 2007; Weyrich et al., 2009).

Platelet membrane phenotype is determined primarily by transmembrane receptors, whose number and state are fundamental for platelet function (Figure 3). These receptors include integrins (αIIbβ3, α2β1, α5β1, α6β1, αVβ3), leucine-rich repeated (LRR) receptors (Glycoprotein Ib-IX-V, Toll-like receptors), G-protein coupled transmembrane receptors (PAR-1 and PAR-4 thrombin receptors, P2Y1 and P2Y12 ADP receptors, TPα and TPβ TxA2 receptors), proteins belonging to the immunoglobulin superfamily (Glycoprotein VI, FcγRIIA), C-type lectin receptors (P-selectin), tyrosine kinase receptors (thrombopoietin receptor, Gas-6, ephrins and Eph kinases) and a variety of other types (CD63, CD36, P-selectin ligand 1, TNF receptor type, etc.). Many of these receptors have been identified in other cell types; some receptors, however, are platelet-specific. The receptors are important for the hemostatic function of platelets, providing specific interaction and a specific functional response to vascular wall adhesive proteins and/or soluble humoral activators. In addition, evidence is being accumulated that some receptors are involved in less-studied platelet reactions, for instance, participation in inflammatory and immune responses, tumor growth, and metastasis of tumor cells (Clemetson & Clemetson, 2013; Goubran et al., 2013).

Glycoprotein IIb-IIIa complex (integrin αIIbβ3) is the best studied platelet receptor. The αIIbβ3 is the only integrin expressed uniquely on platelets and is the major platelet integrin (and receptor) with 50 000 to 80 000 copies per platelet. This protein is always present on platelet plasma membrane. During activation its conformation changes, which can be documented with the use of monoclonal antibodies recognizing the activated or ligand-occupied form of the receptor. Glycoprotein Ib-IX-V complex, with approximately 50 000 copies per platelet, is another most common platelet receptor (after integrin αIIbβ3). GPIb-IX-V is a receptor complex that is always present on the platelet plasma membrane and is responsible for interaction with vWF (Clemetson & Clemetson, 2013). Glycoprotein VI (GPVI) is a member of the immunoglobulin superfamily, expressed exclusively on platelets and megakaryocytes. It serves as the major signaling receptor for collagen, which leads to activation of αIIbβ3 integrin and thrombus formation (Nieswandt et al., 2001). GPVI-mediated collagen responses are receptor density-dependent at the receptor levels expressed on human platelets. There appear to be approximately 3000 copies of GPVI per platelet, and the reduction in the expressed molecule number down to 500 is associated with a loss of responsiveness to collagen and a mild bleeding phenotype (Chen et al., 2002; Dumont et al., 2009). In flow adhesion experiments that closely approximate physiological conditions, GPVI is essential for the formation of large platelet aggregates on collagen. However, GPVI-deficient patients or

mice do not show any severe bleeding tendency. This suggests that a GPVI inhibitor can inhibit thrombus formation, but is not the cause of a significant bleeding tendency (Jung & Moroi, 2008).

The content of α-granules includes both membrane-bound proteins that become expressed on the platelet surface and soluble proteins that are released into the extracellular space (Figure 3). Most membrane-bound proteins are also present on the resting plasma membrane. These proteins include some integrins, immunoglobulin family receptors, leucine-rich repeat family receptors, tetraspanins and other receptors (Berger et al., 1993; Maynard et al., 2007; Niiya et al., 1987; Nurden et al., 2004; Suzuki et al., 2003). However, not all membrane-bound proteins of α-granules are present on non-activated platelets (e.g. the stromal cell-derived factor 1α, P-selectin, CD107a, CD109) (Massberg et al., 2006; Maynard et al., 2007). Some proteins located only in the intracellular granules of resting platelets (e.g. P-selectin) emerge on their surface upon activation. Thus, the presence of P-selectin and other granular membrane proteins on the platelet surface is an indicator of platelet activation.

Recent studies show that cells can compensate their limited abilities to produce specific proteins by receiving them via uptake of circulating microparticles (microvesicles) produced by other cell types (Angelillo-Scherrer, 2012; Owens & Mackman, 2011). Circulating microparticles (MPs) are small fragments of the plasma membrane which are released upon activation and/or apoptosis of vascular cells, including platelets, leukocytes and endothelial cells. The origin of MPs is revealed by detection of antigenic markers specific for parental cells. All MPs are procoagulant since on their surface they contain anionic phospholipids, a substrate for formation of coagulation complexes. Interaction of leukocyte-derived MPs with platelets was demonstrated in model experiments by immune electron microscopy (Rauch et al., 2000) and flow cytometry (Del Conde et al., 2005; Pluskota et al., 2008). The exchange of specific proteins via production and uptake of MPs is an effective tool for coordination of complex cell reactions, such as atherothrombosis or immune response. The uptake of MPs produced upon activation of other cell types enables resting platelets to modify their surface parameters and membrane phenotype.

Platelet activation is associated with a flip-flop move of anionic phospholipids from the inner to the outer leaflet, leading to an increase in these procoagulant phospholipids from 2 to 12% of the total phospholipid content (Figure 3). Exposure of anionic phospholipids on the platelet surface can be monitored by labeling platelets with annexin V, a specific ligand for amino phospholipids (Dörmann et al., 1998). Exposure of anionic phospholipids on platelets provides a catalytic surface for procoagulant processes, enabling thrombin generation at the site of injury (Sims et al., 1989), and affects platelet membrane phenotype.

Role of platelet phenotype in pathophysiology of myocardial infarction

Rapid closure of coronary arteries as a result of occluding thrombus formation is a major cause of acute coronary infarction. In patients with acute ST-segment elevation myocardial infarction (STEMI), fibrin fibers and blood cellular elements are the major components of thrombus formed in acute occlusion of coronary arteries; however, the cellular and fibrin contents of the occlusive thrombus are highly dependent on ischemia time. Fibrin fibers were the major component of thrombus, representing more than 60% of its composition. Platelets, erythrocytes, cholesterol crystals and leukocytes together comprised the remaining 40%. ''Fresh'' thrombi have the highest proportion of platelets ($16.8 \pm 18\%$), while the proportion of fibrin fibers increases over time as the level of thrombin increases, leading to ''older'' fibrin-rich thrombi (Silvain et al., 2011). The role of platelet activation in the pathogenesis of the occlusion process is stressed by a positive correlation between the number of platelets in a forming thrombus and expression of transmembrane glycoprotein CD40, a ligand released by activated platelets ($r = 0.40$, $p=0.02$) (Abu el-Makrem et al., 2009). These results confirm previous *in vivo* animal studies showing the very early stage of thrombus formation, just after endothelial injury, when the initial thrombus is primarily composed of activated platelets rapidly stabilized by fibrin fibers with a decreasing proportion of platelets over time (Balasubramanian et al., 2002; Shand et al., 1987).

The interaction between platelets and injured vascular wall triggers platelet accumulation in the damage site. Subsequent formation of an occluding thrombus strongly depends on the adhesive properties of platelets and their rapid reaction to stimuli emerging in the injured vascular wall (Ruggeri, 2002). These parameters are determined by platelet membrane phenotype, which can be characterized by transmembrane receptors responsible for platelet adhesion and activation in flow. The binding between subendothelial von Willebrand factor and platelet glycoprotein Ib-V-IX is the initial stage of interaction that determines transient adhesion of platelets at the damage site (Savage et al., 1996). Further interaction between adhesion proteins and glycoprotein IIb-IIIa complex (αIIbβ3) leads to firm adhesion. Massberg et al. (2002) showed that inhibition of platelet GPIbα significantly reduced both transient and firm adhesion to the vascular surface of the common carotid artery. In contrast, inhibition of αIIbβ3 had only partial effects on transient platelet adhesion, but almost completely prevented firm attachment to vascular surface *in vivo*. Activation of adhered platelets is determined by glycoprotein VI (GPVI), another platelet-specific glycoprotein whose activity affects the interaction between platelets and collagen fibrils.

Relationships with the intensity of the corresponding platelet reactions were demonstrated for GPIIb-IIIa (Sirotkina et al., 2007), GPI (Baker et al., 2001) and GPVI (Joutsi-Korhonen et al., 2003) variations. Thus, the amount of adhesion molecules could be an important indicator of platelet reactivity. It is established that the number of adhesion molecules on the platelet surface significantly varies in normal individuals and cardiovascular patients: from about two-fold for GPIIb-IIIa and GPIb (Huang et al., 2003; O'Halloran et al., 2006) to five-fold for GPVI (Furihata et al., 2001; Joutsi-Korhonen et al., 2003). However, no differences in GPIIb-IIIa and GPIb numbers on the platelet surface were detected at days 1, 3–5 and 8–12 after onset of myocardial infarction, between acute coronary syndrome

patients and healthy volunteers (Khaspekova et al., 2014). Another platelet-specific glycoprotein that mediates platelet adhesion is GPVI. The amount of GPVI is upregulated on circulating platelets in acute coronary syndrome (Bigalke et al., 2006), and as a consequence, inhibition of GPVI decreases atherosclerosis progression in experimental models (Bültmann et al., 2010).

Histological examination of occluded coronary arteries of patients with acute myocardial infarction (MI) shows increased levels of microparticles (MP) bearing tissue factor (TF). Elevation of procoagulant TF-positive MPs within the occluded coronary artery of patients with MI suggests their pathophysiological role in coronary atherothrombosis (Morel et al., 2009; Palmerini et al., 2013). Generation of these microvesicles by activated leukocytes is well established (Angelillo-Scherrer, 2012; Mallat et al., 2000), while the mechanism of accumulation by occlusive thrombus in general is not known. Acquisition could potentially be mediated by these procoagulant proteins being carried by platelets. Previously, we evaluated the presence of platelets carrying leukocyte membrane marker CD45 in acute myocardial infarction patients. It was found that the fraction of CD45-positive platelets was significantly higher in patients on the first day, and increased further on days 8–10 after MI (Gabbasov et al., 2014). We suggested that platelets gain TF by binding leukocytic MPs, which was confirmed by *in vitro* coincubation of platelets and MPs released from activated granulocytes.

It has become clear that the prototypic acute phase reactant C-reactive protein (CRP) is not only a marker but also a potential active player in the inflammatory process. Being part of the acute phase reaction, plasma levels increase during inflammatory response. Recent evidence suggests that CRP occurs in at least two different conformations: circulating highly soluble native, pentameric CRP (pCRP) and monomeric isoform (mCRP) formed as a result of pCRP dissociation (Wu et al., 2003), representing rather the tissue-bound form of CRP. A physiologically relevant mechanism of anti-inflammatory pCRP dissociation by activated platelets was identified and deposition of pro-inflammatory mCRP in inflamed tissue was demonstrated (Eisenhardt et al., 2009). Molins et al. (2011) have shown that dissociation of blood circulating pCRP into mCRP occurs on shear-induced growing thrombus, and the newly formed mCRP induces further thrombus growth (Figure 1). Furthermore, unlike pCRP, mCRP induces platelet activation and aggregation. Detection of pro-inflammatory mCRP microparticles in the blood of patients following MI confirmed the hypothesis that dissociation of blood circulating pCRP is catalyzed by ''activated'' membranes containing lysophospholipids (Habersberger et al., 2012). These data indicate that monomeric C-reactive protein, which dissociates from pentameric C-reactive protein on the surface of activated platelets, could contribute to atherothrombotic complications in acute coronary syndrome patients by promoting local inflammation.

The chemokine stromal cell-derived factor-1 (SDF-1) is released by activated platelets to recruit bone marrow-derived stem cells at the site of arterial vascular injury, and mediates a P-selectin- and CXCR4-dependent arrest of progenitor cells on matrix-adherent platelets (Zernecke et al., 2005). In experimental studies, the expression of SDF-1 by the platelet-rich thrombus was shown as early as 30 min after vascular injury (Massberg et al., 2006). A later clinical trial has shown significantly enhanced platelet expression of SDF-1 in patients with acute coronary syndrome compared with patients with stable angina pectoris (Stellos et al., 2009).

Clinical impact of platelet phenotype in patients with myocardial infarction

The involvement of platelets in atherothrombotic diseases is unquestioned and investigation of different platelet functional activities in coronary artery disease is an important part of many clinical studies. Several assays are currently available to measure platelet reactivity, and platelet function testing is a valuable tool to assess the effectiveness of antiplatelet drugs. Platelet reactivity testing might become a useful tool to help balance between bleeding and thrombosis with aggressive antiplatelet therapy in acute coronary syndrome (Aradi et al., 2013). High platelet reactivity can be considered a risk factor for patients with MI. The increased hazard associated with high platelet reactivity has been demonstrated by various platelet testing (Franchi et al., 2014). Many researchers attempt to find an optimal method for monitoring platelet reactivity and a ''therapeutic window'', which describes an optimal range of platelet reactivity associated with minimal risk of bleeding and ischemic complications. However, the majority of platelet reactivity assays show only a limited correlation between high platelet activity and ischemic events, and could not predict adverse cardiac events (Tantry et al., 2013).

Tests exploring platelet functional activity in patients with acute coronary syndrome, mainly platelet aggregation, are best clinically validated. Among these tests, the assessment of platelet membrane phenotype is less investigated. Nevertheless, new clinical data indicate that expression of some membrane proteins on the platelet surface may be useful in clinical practice as early additional biomarkers for cardiovascular risk stratification. We will present several new clinical studies which examined the predictive value of two different membrane-associated proteins for acute coronary syndromes to identify patients with a high risk of coronary events.

The first is a prospective study in which the predictive value of platelet surface GPVI for acute coronary syndromes was examined in a large consecutive group of patients with symptomatic coronary artery disease to identify the high-risk cohort with imminent coronary events. A total of 1003 patients with symptomatic coronary artery disease were verified by coronary angiography, and platelet surface expression of GPVI was determined using flow cytometry. Patients with ACS ($n = 485$) showed considerably increased PVI expression compared to patients with stable angina pectoris ($n = 518$). Logistic regression analysis showed that an elevated platelet GPVI level can be indicative of ACS independent of myocardial necrosis biomarkers, including troponin, creatine kinase and creatine kinase-MB. Moreover, patients with increased platelet activation showed significant enhanced residual platelet aggregation despite dual antiplatelet therapy compared to patients with low GPVI levels (Bigalke et al., 2008). In the second study, 1004 patients with chest pain were consecutively examined in a prospective design. ACS was found in 416

(41.4%), stable angina pectoris in 233 (23.2%), and non-coronary (NC) causes of chest pain in 355 patients (35.4%). Patients with ACS showed higher GPVI expression compared to patients with SAP or NC causes of chest pain. Patients with elevated GPVI expression had a poorer clinical outcome than patients with baseline GPVI expression in respect of composite cumulative survival that included myocardial infarction, stroke, and cardiovascular death at three months (Bigalke et al., 2010a). Finally, this group of researchers evaluated 2213 consecutive patients suffering from chest pain in a prospective study. The Kaplan-Meier analysis for cumulative event-free survival revealed that patients with an elevated baseline GPVI expression had a poorer clinical outcome for cardiovascular death than patients with decreased GPVI levels. These results were paralleled in composite cumulative survival that included myocardial infarction, stroke and cardiovascular death (Bigalke et al., 2010b).

Another platelet membrane protein which is included in a large clinical study is stromal cell-derived factor-1 (SDF-1). Expression of this protein is enhanced during ischemic events and may play an important role in trafficking hematopoietic progenitor cells for tissue regeneration and neovascularization. In a prospective study, 1000 patients who were admitted to the emergency department with chest pain were consecutively evaluated. Platelet surface expression of GPIb and SDF-1 was determined by flow cytometry. Patients with ACS showed considerably higher SDF-1 expression on admission compared to patients with stable angina pectoris and chest pain of other origins. Logistic regression analysis showed that surface expression of platelet SDF-1 was significantly associated with ACS. Areas under the curve of receiver operating characteristic analysis revealed 0.718 (95% confidence interval (CI):0.680-0.757) using SDF-1 and 0.795 (95% CI: 0.760-0.829), applying troponin-I baseline serum levels. Patients with higher SDF-1 levels had a 1.4-fold relative risk (95% CI: 1.17-1.52) for ACS (Wurster et al., 2013).

These data indicate that determination of platelet GPVI and SDF-1 can be useful as early additional biomarkers for cardiovascular risk stratification in patients with acute coronary syndrome. Moreover, GPVI can be helpful to distinguish an imminent ACS from non-coronary causes in patients with chest pain who are transferred to a chest pain unit before myocardial necrosis is evident with classical biomarkers.

Genetic polymorphisms and platelet receptors

Genetic variations in $\alpha 2\beta 1$ integrin were reported to be associated with the density of $\alpha 2\beta 1$ receptors on the platelet surface (Kunicki et al., 1997). Namely, an association between genetic variations in $\alpha 2\beta 1$ and the density of $\alpha 2\beta 1$ receptors on the platelet surface was reported. Kunicki et al. (1997) demonstrated that the density of $\alpha 2\beta 1$ receptors on the platelet surface affects platelet adhesion to collagen, contributing to an increased risk of thrombosis.

Both GPIIb and GPIIIa are known to bear a number of single amino acid substitutions affecting conformational changes and the ligand binding function with little or no effect on platelet function. A platelet-specific antigen (PlA1/A2) polymorphism has been by far the most investigated *GPIIIa* gene polymorphism. A substitution of cytosine for thymidine at position 1565 in exon 2 of the *GPIIIa* gene leads to an amino acid difference at position 33: a leucine (A1 allele) or a proline (A2 allele) (Kozieradzka et al., 2007). The above-mentioned polymorphism can influence both platelet activation and aggregation and affect post-occupancy signaling by the platelet fibrinogen receptor IIb/IIIa (Feng et al., 1999; Vijayan et al., 2003). The presence of one or two *PlA2* alleles is associated with an increased binding affinity to fibrinogen as well as with platelet aggregability in response to epinephrine, ADP and collagen *in vitro* (Michelson et al., 2000). It has also been suggested that the *PlA2* allelic variant causes increased sensitivity to platelet aggregation by various agonists and altered sensitivity to aspirin (Bajt et al., 1994; Cooke et al., 2006; Szczeklik et al., 2000).

Conclusion with perspectives

The platelet is a complex cell packed full of active molecules and metabolites and is able to rapidly change native phenotype upon activation. It is well established that increased platelet activation can contribute to atherothrombosis and inflammation in patients with acute coronary syndrome. The negative effect of platelets can be reduced through antiplatelet therapy by inhibiting their pro-thrombotic and pro-inflammatory properties. These properties are strongly determined by platelet phenotype, which can be described by surface expression of different types of membrane-associated proteins. Among these we can highlight glycoprotein receptors and integrins (GPIb, GPVI, $\alpha IIb\beta 3$, $\alpha 2\beta 1$, etc.), procoagulant proteins (lysophospholipids, coagulation factors), cell adhesion molecules (selectins, fibrinogen, fibronectin, vWF, etc.), chemokines (SDF-1) and some membrane-associated pro-inflammatory proteins (mCRP). Membrane-associated proteins that mediate thrombosis and inflammation might represent hypothetically ideal markers of their functional status. For example, for ACS patients remaining on clopidogrel, platelet reactivity testing seems to be of crucial importance due to the variable and unpredictable effect of clopidogrel. Platelet phenotype identification to assess the level of P2Y12-receptor inhibition may play a key role in choosing the best drug for the individual patient.

An ideal and reliable platelet activation biomarker should be cheap, usable away from specialized laboratories, informative and specific in order to orientate the physician towards diagnosis or predictable outcome. Moreover, a test for monitoring antiplatelet drugs should be validated not only in terms of discriminating patients at risk, but in terms of demonstrating that alternative treatment regimens are effective. Unfortunately, unlike myocardial injury markers such as troponins, and despite the fact that several biomarkers of *in vivo* platelet activation have been suggested, none offers ideal diagnostic properties (Ferroni et al., 2012; Harrison, 2012). For example, platelet phenotype description requires a complex, time-consuming sample preparation, the use of a flow cytometer, and an experienced technician.

Irrespective of differences in the determination of membrane-bound proteins whose levels are strongly associated with disease diagnosis, therapy and prognosis, these determinations are possible, albeit on a limited scale. Today, platelet phenotyping can be used for better diagnosis or in

combination with reflection of distinct pathophysiological features of ACS, such as cell necrosis, vascular inflammation or oxidative stress that significantly improve our ability to identify patients who are at risk of adverse cardiovascular events (De Filippi & Seliger, 2009). Large-scale implementation of these technologies requires simple and informative methods for point-of-care testing that allows cell activity monitoring without highly qualified personnel. This will need to be validated by extensive clinical trials. Nevertheless, there are experimental prerequisites for these methods.

Declaration of interest

The authors declare no conflict of interest.

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