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Review Article

Blood platelet biochemistry ☆

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ABSTRACT

Defects in platelet function or formation increase the risk for bleeding or thrombosis, which indicates the crucial role for platelets in maintaining haemostasis in normal life. Upon vascular injury, platelets instantly adhere to the exposed extracellular matrix which results in platelet activation and aggregation and the formation a haemostatic plug that stops bleeding. To prevent excessive platelet aggregate formation that eventually would occlude the vessels, this self-amplifying process nevertheless requires a tight control. This review intends to give a comprehensive overview of the currently established main mechanisms in platelet function.

Contents

Introduction

Although platelets are involved in different processes such as triggering inflammation, fighting microbial infection, promoting tumor metastasis, and embryonic blood/lymphatic vessel separation [1], their principal function still remains stopping hemorrhage following vascular injury. Upon tissue trauma platelets initially tether and roll over the exposed extracellular matrix, a process that eventually results in firm platelet adhesion and that triggers a signaling cascade mediated by tyrosine kinases and G-protein coupled receptors, resulting in full platelet activation with concomitant granule release. Released effectors in turn recruit and activate additional platelets, next leading to platelet aggregation and the presentation of a procoagulant surface promoting formation of a fibrin-rich haemostatic plug. Platelet activation, in addition, also triggers endothelial cells to locally synthesize and secrete molecules that limit thrombus formation.

Platelet adhesion

As a first response to vascular injury, platelets immediately adhere to the exposed subendothelial extracellular matrix. This matrix contains several ligands for different platelet receptors, including

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collagen, von Willebrand factor (VWF), laminin, fibronectin and thrombospondin. Since fibrillar collagens type I and III are very effective platelet activators and have a high affinity for VWF, they are considered to be the most thrombogenic matrix-mediators of platelet adhesion. [2]

Depending on the rheological conditions, platelet adhesion occurs via different mechanisms. Especially at high shear rates, VWF is essential to decelerate fast-flowing platelets by reversible binding of its A1 domain with platelet glycoprotein (GP) Ibα. This initial platelet tethering allows the establishment of additional interactions between platelets and the subendothelial matrix, e.g. engagement of the collagen receptors GPVI and α2β1. This eventually leads to definitive platelet arrest and subsequent platelet thrombus formation. Optimal platelet adhesion relies on synergistic actions between the different platelet receptors. For example, both collagen receptors reinforce each other's activity [3], but also α2β1 and GPIbα cooperate synergistically, further reinforced by activation via GPVI [4,5].

GPIbα -VWF

VWF is a large, multimeric glycoprotein that is present in plasma, the subendothelial matrix and storage granules in both platelets (α-granules) and endothelial cells (Weibel-Palade bodies). [6] Upon injury of the vessel wall, circulating VWF rapidly binds to exposed collagen through collagen binding sites that are present in the VWF A1 and, more importantly, VWF A3 domain [7,8]. Self-association between circulating VWF multimers and matrix-bound [9], platelet-bound [10] or endogenous subendothelial VWF is also possible and involves multiple domain interactions [11].

After immobilization, VWF undergoes conformational changes that expose the binding site in its A1 domain for GPIbα [12-14]. GPIbα, the only receptor on a non-activated platelet with a significant affinity for VWF, is part of the GPIb/IX/V complex, which consists of the leucine-rich repeat glycoproteins GPIbα (130 kDa), GPIbβ (25 kDa), GPIX (22 kDa) and GPV (88 kDa) [15] in a 2:4:2:1 stoichiometry.

The transient interactions between VWF and GPIbα allows 'rolling' of platelets on sites of vascular injury, keeping them in close contact with the exposed subendothelial matrix. As a result, other platelet receptors, such as those for collagen, are engaged, which leads to eventual platelet activation and firm platelet adhesion via high affinity β1 and β3 integrins. Activated platelets are then crosslinked by binding of VWF to GPIbα under high shear and of VWF and fibrinogen to the integrin αIIbβ3 once activated. Patients with deficiencies in either VWF (von Willebrand disease [6]) or GPIbα (Bernard-Soulier Syndrome [16]) suffer from severe bleeding symptoms, underlining the key role of the VWF-GPIbα interaction in normal haemostasis.

GPIbα has multiple binding partners, including α-thrombin (FIIa) [17] which engages the receptor through a two-site mechanism recently described [18]. Despite improved molecular insights, it remains unclear what major anti- or prothrombotic effect this interaction has both on α-thrombin and GPIbα. Nevertheless, in experimental thrombosis models, it is clear that thrombosis is much more impaired in mice lacking the extracellular part of GPIbα than in mice lacking VWF [19]. This suggests that besides VWF, also the other GPIbα ligands (e.g. thrombospondin-1) can mediate thrombus formation.

Platelet collagen receptors GPVI and α2β1

GPVI (62 kDa) is a platelet-specific member of the immunoglobulin superfamily, consisting of two extracellular immunoglobulin-like domains, a mucin-like stalk, a transmembrane region and a short cytoplasmic tail [20]. In the platelet membrane, GPVI is associated with the FcRγ-chain, which bears immunoreceptor tyrosine-based activation motifs (ITAM) for signal transduction [21]. The role of GPVI is considered to be eliciting strong signaling rather than establishing stable platelet adhesion [5]. Indeed, GPVI has only a low affinity for collagen (involving the recognition of triplets consisting of Gly, Pro and Hyp [22-24]) whereas GPVI/FcRγ-chain-mediated signaling is crucial for platelet adhesion on collagen [25].

α2β1 (GPIa/IIa, VLA-2 or CD49b/CD29) is an I-domain containing integrin with a high affinity for the Gly-Phe-Hyp-Gly-Glu-Arg sequence in collagen [26]. Efficient collagen binding depends on activation of α2β1, which shifts the conformation of this integrin from a low-affinity to a high-affinity state. An intermediate state of α2β1 has also been reported [27-29]. The precise signaling pathway leading to α2β1 activation is not yet entirely elucidated but involvement of GPVI and/or prior activation of αIIbβ3 have been suggested [30-32].

Platelet activation (Fig. 1)

A major signal transduction pathway by which platelets are activated involves a cascade of tyrosine kinases, as occurs when GPIb is occupied by VWF [33], the FcRγII receptor by antibody complexes, the receptors Tyro3, Axl and Mer by GAS6 [34], GPVI-FcRγ by collagen [35] or CLEC2 by podoplanin, the latter essential for the separation of the lymphatic system from the blood vessels in embryonic development [36].

When GPVI is crosslinked and clustered by collagen (or a collagen-related peptide, the snake C type lectin convulxin, or antibodies) [20] this leads to activation of the Src tyrosine kinases Fyn and Lyn, bound to the cytoplasmatic tail of GPVI. The ITAMs present on the FcRγ-chain are phosphorylated by Fyn and Lyn, next allowing the recruitment of the tyrosine kinase Syk. Syk in turn induces a signaling cascade finally resulting in the Tyr-phosphorylation and activation of phospholipase Cγ2 (PLCγ2), for which in addition both the adaptor protein SLP-76 and "linker for activation of T cell" (LAT) are required [35].

Activated PLCγ2 hydrolyzes phosphatidylinositol 4,5 bisphosphate to produce inositol 1,4,5 trisphosphate (IP_3) and membrane bound 1,2-diacylglycerol (DAG). IP_3 rapidly diffuses and binds to its receptor IP_3R, a calcium-selective channel on the platelet dense tubular system (DTS), through which now an efflux of Ca^{2+} from the DTS starts with increasing Ca^{2+} levels in the cytoplasm.

Stromal interaction molecule 1 (STIM1) equally is a DTS transmembrane protein, which contains a Ca^{2+} binding EF hand motif in its DTS region, which in resting platelets, is occupied by Ca^{2+}. However when Ca^{2+} levels within the DTS are reduced following IP_3R activation, STIM1 is no longer occupied by Ca^{2+} and translocates to the plasma membrane where it associates with and opens the storage operated calcium channel Orai1, allowing influx of plasma Ca^{2+} entry in activated platelets with an even further increase in cytosolic calcium [37,38].

The hydrophobic DAG in the membrane together with Ca^{2+}, bound to phosphatidylserine, induces the translocation of the serine/threonine protein kinase C (PKC) to the membrane, by which it becomes activated to play a prominent role in the secretion reaction.

Raising cytosolic Ca^{2+} and DAG concentrations within the adherent platelet cytosol results in a plethora of reactions including phospholipase A2 (PLA2) activation, platelet shape change, granule secretion and finally aggregation. The Ca^{2+}-increase is counterbalanced by plasma membrane Ca^{2+}ATPases (PMCAs) that extrude cytosolic Ca^{2+} towards the extracellular medium and sarco/endoplasmic reticulum Ca^{2+}ATPases (SERCAs) that pump Ca^{2+} into the DTS [39].

Increased cytosolic Ca^{2+} levels also is responsible for the exposure of negatively charged phosphatidylserine at the platelet surface [40] due to activation of a scramblase, recently identified as the transmembrane protein 16 F (TMEM16F) [41]. This negatively charged procoagulant surface provides together with bound Ca^{2+}, binding sites for especially the vitamin K-dependent clotting factors, co-factors and their substrates, which accelerate the coagulation cascade. In addition, during this process also procoagulant microparticles are formed [42]. The importance of the contribution of the procoagulant surface is