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Different Stages of Platelet Adhesion to the Site of Vascular Injury

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Abstract

Platelet activation and adhesion to the site of vascular injury is a dynamic process comprising reversible and irreversible phases. Platelet adhesion typically occurs in a multi-step process similar to the selectin/integrin-mediated adhesion of neutrophils. This phenomenon is highly regulated and influenced by the cross-talk between platelets and injured endothelium. This cross-talk involves a variety of mediators including adhesion molecules and receptors, agonists, chemokines, shed proteins and various proinflammatory lipids.

This review briefly discuses the main adhesion molecules and receptors involved in both reversible and irreversible phases of platelet adhesion to the site of vascular injury, leading to a better characterization of the multistep mechanisms of thrombus formation.

Keywords: Platelet, adhesion molecules, procoagulant activity, thrombosis

Introduction

Platelets are small anucleate cells that are derived from cytoplasmic fragments of bone marrow megakaryocytes. Resting platelets have a flat, featureless discoid morphology that is ideally suited for their circulation through the microvasculature [1,2]. The mean size of platelets varies amongst individuals, ranging from approximately 1.5 to 3.0 µm in diameter and appears to follow a log normal distribution [3]. The platelet plasma membrane is composed of a bilayer of phospholipids consisting of cholesterol, glycolipids and glycoproteins including functional receptors and ligands [4]. The negatively charged phospholipids are almost exclusively present in the inner leaflet of resting platelets [5], however following platelet activation by potent agonists, these aminophospholipids are exteriorized and are able to accelerate several steps in the coagulation reaction, ultimately generating thrombin. Thus the presence of these lipids in the inner leaflet of resting platelets is considered a control mechanism to prevent inappropriate coagulation activation [6-8].

Platelets typically circulate in the bloodstream for 7-10 days and their principal function is to survey the inner lining of blood vessels to detect breaches in the vasculature. They rapidly adhere to sites of blood vessel injury, aggregate with one another and facilitate the generation of thrombin. These events are critical for the normal haemostatic process. Primary platelet adhesion and aggregation leads to the formation of a platelet plug (termed the primary haemostatic plug), while subsequent thrombin generation and the conversion of soluble fibrinogen to fibrin acts to consolidate the primary haemostatic plug (termed secondary haemostasis) and stop bleeding. Platelet activation and adhesion to the site of vascular injury typically occur in a multi-step process similar to the selectin/integrin-mediated adhesion of neutrophils. This is a dynamic process comprising reversible and irreversible phases which is tightly regulated to avoid excessive platelet accumulation at the injury site and vascular obstruction, the principal pathological process causing heart attacks and ischaemic stroke. This review discusses aforementioned process, with respect to its distinct multi-step stages, to afford a clear picture into the complex molecular mechanisms of primary events in thrombus formation.

Platelet adhesion

Macromolecular adhesion proteins

von Willebrand factor (vWf)

vWf is an adhesive multimeric glycoprotein which contains binding sites for numerous proteins, including platelet membrane glycoproteins and constituents of the subendothelial matrix. vWf is synthesized in endothelial cells [9] and in megakaryocytes [10, 11]. In endothelial cells, vWf is packaged into secretory vesicles known as Weibel-Palade bodies where its release is tightly regulated. In addition, the protein is constitutively secreted from the basal side of the endothelial cell where it becomes part of the subendothelial matrix [12]. In megakaryocytes, vWf is packaged into α-granules where it is released upon platelet stimulation [13, 14]. The protein contains several important domains that enable it to interact with other subendothelial proteins and platelet receptors. The A1 domain is possibly the most important functional part of the molecule since it contains binding sites for GPIbα, collagen, sulphatides and heparin [15,16]. The carboxyl-terminal end of individual vWf subunits (C1 domain) contains an arginine-glycine-aspartic acid (RGD) sequence that is important for its interaction with activated integrins, in particular platelet integrin αIIbβ3 [17-19].

Fibrinogen

Fibrinogen circulates as a dimer made up of three polypeptide chains Aα, Bβ and γ which are disulphide linked (20). Similar to vWf, fibrinogen is found in two pools, in plasma and platelet α-granules. However unlike vWf, megakaryocytes do not appear to synthesize fibrinogen; rather it is taken up from plasma and concentrated in the α-granules by a process that involves the integrin αIIbβ3 receptor [21]. The dodecapeptide sequence (HHLGGAKQAGDV) on the carboxyl-terminal end of the fibrinogen γ chain is the most important binding site for integrin αIIbβ3 [22-24]. Other αIIbβ3 binding sites that have been identified on the fibrinogen molecule include two RGD sequences on the Aα chain of each monomer. This is a recognized motif common amongst adhesive proteins important for binding to integrins. In soluble fibrinogen, the RGD sequences lie within the triple helical coiled domain without direct exposure to integrin αIIbβ3. However, when fibrinogen is immobilized to a surface, exposure of at least one of these sequences allows it to bind the integrin αIIbβ3 in its inactive conformation [25].

Collagen

Collagen is a major constituent of the vessel wall and provides a highly reactive surface for platelets, thereby promoting rapid thrombus formation [26]. Adhesion of platelets to collagen under high shear conditions is dependent on collagen-bound vWf [27-29]. The interaction between collagen-bound vWf and the platelet receptor GPIb/V/IX is critical to initiate platelet adhesion under rapid blood flow conditions, slowing platelet movement at the vessel wall to enable receptor-ligand interactions with slower-binding kinetics, such as the collagen-GPVI or collagen-integrin α2β1, to promote platelet arrest [30].

At least 25 types of collagen have been identified [31] of which types I, III, IV, V and VI are the most abundant in the vascular wall [32]. Collagen types I-IV are highly reactive for platelets [33] supporting adhesion and aggregate formation under low shear conditions. Other collagens vary considerably in their ability to support platelet adhesion and activation, and their pathophysiological importance in mediating these events is unclear [33, 34].

Adhesion receptors

The GPIb-V-IX complex

This receptor complex belongs to the leucine-rich glycoprotein family of adhesion receptors and serves as the primary receptor for vWf [35, 36]. Each receptor complex is comprised of four separate glycoproteins arranged in a specific order. GPIb is a heterodimer of a larger α chain (GPIbα) and a smaller disulfide linked β chain (GPIbβ) which forms a non-covalent complex with GPIX. Two GPIb/IX complexes are linked together by GPV forming the complete receptor [37]. GPIbα is the largest and the most important part of the complex and contains the binding site for vWf [38]. In arterioles where the shear rates are high, the initial capture of platelets on sub-endothelial collagen is mediated by collagen bound vWf interacting with GPIb-V-IX. On the other hand, on a developing thrombus additional platelets are recruited by the interaction of GPIb-V-IX on flowing platelets, with vWF bound to activated integrin αIIbβ3, on the surface of the thrombus [30, 39-41]. The binding of VWF to GPIb-V-IX induces platelet signaling that results in the upregulation of integrin

αIIbβ3 affinity [42-47]. This enables the integrin to bind VWF and fibrinogen thereby enhancing platelet adhesion and thrombus formation.

Collagen receptors

Integrin α2β1 was the first platelet collagen receptor to be identified [48-51]. The binding of collagen to this receptor has not been reported to stimulate tyrosine kinase activity, which is required for collagen-induced platelet activation. It has been proposed that this receptor, by anchoring the platelet to collagen, facilitates the interaction between collagen and a second receptor (GPVI) leading to cell signaling and platelet activation [52]. This model, in which platelet adhesion and activation are distinct events, is supported by more recent studies [53-56].

GPVI belongs to the immunoglobulin receptor superfamily [57,58], and is non-covalently associated with FcR γ-chain, which serves as the signal-transducing component of the receptor [59, 60]. Studies on GPVI knockout mice support the hypothesis that GPVI is the dominant collagen receptor inducing platelet activation [61-64].

Integrin $\alpha_{IIb}\beta 3$

Integrins are heterodimeric glycoproteins consisting of an α subunit complexed to a β subunit through non-covalent binding. At least 8 different β subunits and more than 16 different α subunits have been identified. Integrins on human cells are categorized by the β subunit of the heterodimer (i.e., β1 or β2 integrin subfamilies) [65, 66]. They mediate cell adhesion to extracellular matrix proteins and to other cells, as well as transmitting signals from the extracellular environment. These are essential for cell motility, growth and differentiation, cell survival, and specialized functions such as degranulation and oxidant production in immune cells [67-71].

Platelets express several integrins on their surface and of these integrins, αIIbβ3 is functionally the most important and most extensively studied. Integrin αIIbβ3 is the most abundant of all the platelet surface protein receptors and its expression is restricted to platelets and megakayocytes [72, 73]. It plays a critical role in platelet adhesion, aggregation and spreading and its physiological importance is highlighted by the observation that individuals with Glanzmann's Thrombasthenia, where the receptor is either absent or defective, have significant bleeding problems [74,75]. Platelets from these patients are unable to aggregate in response to multiple physiological stimuli. Like other integrins, αIIbβ3 can exist in multiple stages of activation and its ligand binding function is tightly regulated. Cell signaling converts the integrin from a low to a high affinity receptor which enables it to engage its ligands, including fibrinogen, vWf, vitronectin and fibronectin through an RGD recognition sequence present on all these proteins [76-78].

Mechanism of platelet adhesion to reactive surfaces

Platelet tethering

Platelet adhesion typically occurs in a multi-step process similar to the selectin/integrin-mediated adhesion of neutrophils. GPIb-V-IX plays a critical role in capturing free-flowing platelets to the vessel wall, its rapid binding kinetics enables platelet tethering even under conditions of high shear through an interaction with subendothelial-bound vWF. However, the accompanying rapid dissociation rate means this interaction supports platelet translocation and is therefore unable to maintain the cell in a state of stationary adhesion [39]. During translocation on vWf, platelets undergo rapid morphological changes characterized by transition from a flat disc to a sphere with multiple filopodial protrusions [47]. This is a critical period during which one of two scenarios may occur: 1) the platelet may detach and return to the circulation, or 2) it becomes activated leading to irreversible adhesion and thrombus formation.

Stable adhesion

The vWf/GPIb-V-IX interaction slows platelet velocity in the rapidly flowing blood allowing other receptors with slower binding kinetics to bind their specific ligands. Meanwhile, platelet activation contributed by multiple adhesion receptors and soluble agonists induces intracellular signaling mechanisms leading to the conversion of integrin αIIbβ3 from a low affinity to high affinity state. This enables the integrins to bind ligand and mediate stationary adhesion. Stationary adhesion is also critically supported by the binding of collagen receptors (GPVI and α2β1) to collagen in the subendothelium [33]. Cell activation is further enhanced through production of TXA2 and soluble

agonists such as ADP, thrombin and adrenaline, which bind to their respective receptors on the platelet surface. The majority of these receptors are linked to heterotrimeric G-proteins that serve to initiate an efficient inside-out signaling pathway in platelets, causing further integrin $\alpha_{IIb}\beta3$ activation with the resultant enhancement in platelet adhesion and aggregation [79].

In response to potent soluble agonists, the platelet α-granules release their contents which are either shuttled to the plasma membrane or secreted to the extracellular milieu. This process is important in facilitating the development of platelet aggregates ($\alpha_{IIb}\beta3$ and fibrinogen), wound healing (platelet derived growth factor), thrombin formation (factor V) or platelet–leukocyte interaction (P-selectin and CD40L) [80, 81].

Integrin $\alpha_{IIb}\beta3$ activation: linking platelet aggregation to thrombus formation

Activation of integrin $\alpha_{IIb}\beta3$ is tightly regulated through a process termed inside-out signaling. Agonists, such as ADP and thrombin, which engage GPCRs, or adhesive proteins, such as collagen or vWF, which interact with GPVI or GPIb-IX-V respectively, initiate intracellular signaling events that influence integrin affinity by modifying the cytoplasmic tail of αIIb and or β3. These modifications propagate to the extracellular domain of the receptor resulting in alteration in the conformation of the ligand binding site and imparting binding competency to the integrin (review by Shattil et al 1998). Structural and crystallographic studies have proposed that in a low- affinity state, the ligand binding site of integrin is cryptic and becomes exposed following

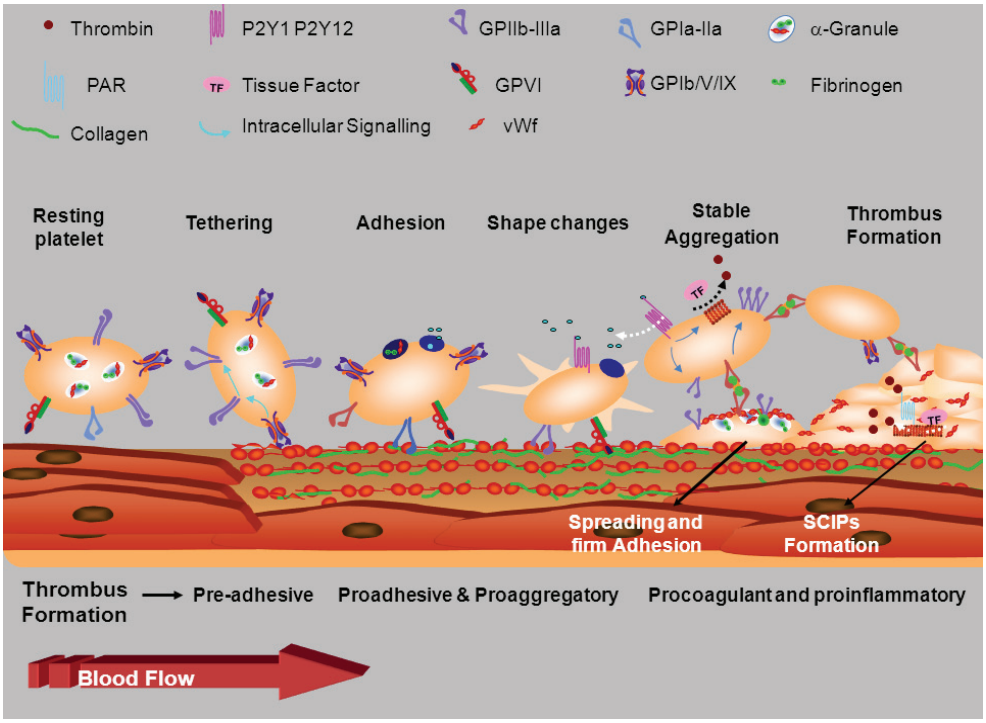

Figure 1: Transformation of platelets from a proadhesive, proaggregatory to procoagulant and proinflammatory state under physiological flow conditions. Platelets are initially captured from free flowing blood through the binding of GPIb/V/IX to immobilized vWF expressed at sites of vascular injury. This interaction slows down platelet movement and allows GPVI to engage the exposed collagen. These interactions then trigger the activation of the major integrin αIIbβ3 which facilitates platelet stationary adhesion, spreading and subsequent aggregation through binding to vWf and fibrinogen. Activated platelets release granule stored ADP which promotes further platelet activation and thrombus growth through its receptors P2Y1 and P2Y12. The sustained calcium elevation within activated platelets results in the surface exposure of phosphatidylserine (PS) and transforms platelets from a proaggregatory to pro-coagulant state. The exposed PS initiates the generation of thrombin which through binding to PARs reinforces platelet activation and thrombus growth. In concert with other platelet stimuli such as collagen, thrombin induces Sustained Calcium Induced Platelet (SCIP) morphology which is associated with the specific production of inflammatory lipids to promote procoagulant and proinflammatory function of platelets.

conformational changes in the β3 cytoplasmic tail [82-84]. Based on current data, integrin activation at the cytoplasmic tail involves a clasping/unclasping mechanism which provides the driving force to trigger the conformational changes in the extracellular domain [85]. It is also known that the cytoplasmic domains of the receptor are critical for affinity modulation, as mutations in certain positions of the cytoplasmic tail can result in defective or enhanced activation of the integrin [86-89]. Integrin affinity modulation induced by inside-out signaling is critical for receptor activation and for the engagement of soluble ligands. Cross-linking of the receptor followed by the binding of activated integrins to multimeric ligands (e.g. fibrinogen and vWF), induces outside-in signaling pathways which trigger the clustering of integrins within the plane of the plasma membrane. This process enhances substrate-binding avidity and signaling capacity [90], leading to irreversible phases of platelet activation. In this phase fully activated platelets covered by clustered integrins become strongly cross-linked together through the ligation with fibrinogen molecules, resulting to initial phase of thrombus formation [91, 92].

Thrombus formation

The firm adhesion of platelets to the site of injury forms a monolayer which serves as a reactive site for further recruitment of free-flowing platelets. Platelet accumulation is dependent on platelet-bound vWf and fibrinogen which serves to cross-link adjacent platelets and promote stable platelet aggregation as [91, 92]. This process is essential for the formation of a primary haemostatic plug and also for the development of pathological thrombi at site of atherosclerotic plaque rupture. During thrombus development, the sustained calcium elevation within activated platelets results in the surface exposure of phosphatidylserine (PS) and transforms platelets from a proaggregatory to pro-coagulant state. The exposed PS initiates the generation of thrombin, which through binding to PARs, reinforces platelet activation and thrombus growth. In concert with other platelet stimuli such as collagen, thrombin induces Sustained Calcium Induced Platelet morphology (SCIP) which is associated with the specific production of inflammatory lipids to promote procoagulant and proinflammatory function of platelets [93]. Figure.1 demonstrates different steps thrombus develoment including platelet tethering, adhesion, aggregation, thrombus formation and procoagulant activation.

Conclusion

Over last three decades, the molecular mechanisms underlying platelet activation and adhesion have been increasingly well defined, particularly with respect to the key factors involved in the multi-step mechanisms of thrombus development. These achievements have been obtained thorough continuing advances in in vitro and in vivo experimental systems, combined with the development of genetically manipulated mouse models. However, better understanding of precise mechanisms regulating these events and how such well preserved conditions lead to an uncontrolled pathophysiological status, still remain the main interest of scientists in their current and future investigations to control and diminish the risk of cardiovascular diseases.

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