Platelet biology – an overview

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SUMMARY

Understanding the biology of platelet is important for one to treat patients with acquired or inherited platelet disorders. Platelets provide primary hemostasis by performing five primary functions: adhesion, aggregation, secretion, and providing procoagulant surface and clot retraction. The elucidation of the molecular mechanisms carrying out these functions has led to a better understanding of the pathophysiology of bleeding and the development of new approaches in treating patients with qualitative and quantitative platelet disorders. The purpose of this review is to provide a foundation for understanding the other papers in this publication on the treatment of platelet disorders.

HEMOSTASIS

Hemostasis is the complex physiologic process that leads to the arrest of bleeding. It consists of three components: (i) platelets; (ii) plasma proteins; (iii) blood vessels and endothelial cells.1

With injury to a vessel, the forces of all three components are quickly brought to bear. Blood vessels constrict, platelets aggregate to form a plug, the plug is stabilized by the formation of insoluble fibrin due to the action of thrombin on soluble fibrinogen.

Blood fluidity is maintained by checks and balances: blood coagulation is normally held in check by a series of enzymes comprising the fibrinolytic pathway, which restores vascular patency. A complex series of anticoagulants also inhibit the formation of intravascular thrombin. Disruption of these balances leads to clinical bleeding or thrombus formation (Figure 1).2

Platelets

Platelets are produced in the bone marrow megakaryocytes stimulated by the growth factor thrombopoietin. The normal platelet concentration is 150,000–350,000/μL. Platelets circulate in the blood for 10 days.3

OVERVIEW OF PLATELET FUNCTIONS

Adhesion

The adhesion molecule von Willebrand Factor (vWF) binds to subendothelium which then allows platelets to
adhere to the injury site, and occurs within 1–3 seconds after an injury.  

**Secretion**

*Intracellular signaling (‘platelet activation’)*

A variety of platelet agonists, including thrombin generated from the clotting cascade, epinephrine, collagen from the subendothelium, and ADP, cause intracellular signaling leading to secretion.

Platelets have at least five functionally different guanine nucleotide-binding proteins, or G proteins. Once an agonist-like thrombin binds to its receptor, its associated G protein releases GDP, binds GTP and activates a signal-generating enzyme, such as phospholipase C. Phospholipase C hydrolyzes the precursor inositol phospholipid PIP$_2$ into the second messengers inositol 1,4,5-triphosphate (IP$_3$) and 1,2-diacylglycerol (DAG). IP$_3$ causes an increase in intracellular calcium and DAG activates protein kinase C (PKC) and these molecules effect secretion, shape change and aggregation.  

*The ‘secrete’ life of a platelet*

Upon activation, platelets secrete the contents of the dense granules (DG) which contain calcium, ADP, ATP, GTP, thromboxane and serotonin. Alpha granules (AG) fuse to the plasma membrane and release their contents upon platelet activation. These granules contain the adhesive molecules, fibrinogen, vWF, thrombospondin and fibronectin, which promote additional adhesion and aggregation. ADP promotes more platelet activation and thromboxane A$_2$ promotes blood vessel constriction. With secretion shape changes leading to pseudopodia occur which facilitate aggregation to other platelets.  

**Aggregation**

Platelets bind to each other. Formation of this platelet ‘web’ is the result of the binding of fibrinogen to glycoprotein IIb–IIIa, an integrin molecule that only binds fibrinogen after the molecule undergoes a conformational change, the result of the above described platelet activation processes.

The amino acid sequences of GPIIb and GPIIIa helped define a family of adhesion molecules that have come to be known as integrins. Integrins are a family of structurally related glycoproteins that consist of alpha and beta subunits, and bind adhesive molecules (like fibrinogen). The term ‘integrin’ was coined to refer to an integral membrane protein that served as a bridge between the extracellular matrix and the cytoskeleton of the cell.

GPIIb–IIIa belongs to a subclass of integrins characterized by their ability to bind ligands containing the amino acid sequence: arginine-glycine-aspartic acid-serine (RGDS). Fibrinogen is rich in RGDS sequences.  

**Platelet procoagulant activity**

The activated platelet membrane permits the binding of clotting proteins Factor V and X complex, leading to greatly increased catalytic activity of these plasma factors. It does this by providing phospholipid binding sites.

The above takes 3–4 minutes, and can be measured in a patient by doing a bleeding time (a simple test done by making a small incision on the forearm, and measuring how long it takes to form a clot).

These processes can be summarized as in (Figure 2).
Platelet aggregation (Figure 5)

Platelet adhesion to other platelets is referred to as platelet aggregation and is to be distinguished from platelets interacting with the subendothelium. Aggregation requires the binding of fibrinogen to its receptor on activated platelets. In the resting state, fibrinogen membrane seven times with a long amino terminus exterior to the cell and a short intracellular terminus. Thrombin activates its receptor in a unique function by cleaving a portion of the distal end of the receptor generating a new amino terminus which in turn interacts with another region of the receptor to generate is activation signal. To date no deficiency in the thrombin receptor has been identified.¹⁰

Platelet secretion (Figure 4)

The most potent of these includes thrombin, which belongs to a family of molecules that span the plasma membrane seven times with a long amino terminus exterior to the cell and a short intracellular terminus. Thrombin activates its receptor in a unique function by cleaving a portion of the distal end of the receptor generating a new amino terminus which in turn interacts with another region of the receptor to generate is activation signal. To date no deficiency in the thrombin receptor has been identified.¹⁰

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Figure 5. Overview of platelets aggregation to other platelets by fibrinogen binding to platelet glycoprotein IIb–IIIa.

Figure 6. Overview of normal platelet function. PLC, PIP$_2$, PLA$_2$, MLC, PGH$_2$ represent steps in thromboxane synthesis. ADP, adenosine diphosphate; PLT, platelet; RG, receptor and G linked proteins; TBX-A, thromboxane.

PATHOPHYSIOLOGY OF QUALITATIVE PLATELET DISORDERS

Quantitative platelet disorders are molecular lesions of the above described molecules leading to platelet dysfunction, inherited or acquired (Figure 6).$^{12}$

Inherited disorders of platelet function

**Aggregation**

The most common inherited disease of platelets is Glanzmann thrombasthenia. This is an autosomal recessive disorder characterized by mucocutaneous bleeding of mild-to-moderate severity from the time of birth. The platelet count is normal, the platelets appear normal, the bleeding time is prolonged and platelet aggregation to all physiologic stimuli is absent. The vast majority of cases are due to virtual absence of the GPIIb–IIIa fibrinogen receptor. Because both subunits are required for assembly and surface expression, the absence of one glycoprotein results in the eventual intracellular degradation of the unpaired partner.$^{13}$

**Adhesion**

Inherited defects leading to absence of the GPIbα protein are seen in classical Bernard–Soulier syndrome. These patients bleed because of an inability to bind vWF and hence, adhere to subendothelium. Often they have a slightly low platelet count and large platelets on the peripheral blood smear.$^{14}$
**Secretion**

The storage pool diseases are characterized by a lack of DG, AG, or a specific component of one of the granules (classically, ADP). Bleeding is mild-to-severe and *in vitro* platelet aggregation studies are diminished.\(^\text{15}\)

**Acquired disorders of platelet function**

Drugs, toxins built up during renal failure, physical trauma when the blood is exposed to extracorporeal circulation in heart surgery, dietary agents (omega 3 fatty acid from fish oil) may all interfere with platelet function.\(^\text{16}\)

Aspirin is a common non-prescription drug affecting platelet function. The basis of this is as follows (Figure 7):

**PLATELET HISTOLOGY**

Platelets are not true cells, but fragments of megakaryocytes. The drawing below shows the canalicular system (CS) that permits the ingress and egress of molecules needed for hemostasis and transport. The DG contain serotonin, ADP, ATP and thromboxane. The AG release fibrinogen, and vWF. There are abundant mitochondria (M).\(^\text{17}\)

An extensive system of microtubules (MT) maintains the normal, resting discoid shape of the platelet. Upon activation, platelets demonstrate a remarkable change in morphology, changing from the discoid shape to a smaller, contracted sphere with long filamentous pseudopodia. This provides up to 50% additional surface area for contact with other platelets and subsequent clot retraction (Figure 8).

**PLATELET–ENDOTHELIAL INTERACTIONS** (Figure 9)

Under normal conditions platelets do not adhere to endothelial cells. The non-thrombogenic nature of endothelial cells is due to both passive factors (a negative charge on its surface) and numerous active processes. Prostacyclin (also called PGI\(_2\)) is a vasodilator and inhibits platelet activation by raising cyclic AMP levels. Endothelial cells also release endothelial-derived releasing factor (EDRF) or nitric oxide (NO) which can inhibit platelet function.\(^\text{18}\)

In addition, tissue plasminogen activator (tPA) promotes lysis of clots. Thrombomodulin (TM) serves to inactivate clotting proteins by inhibiting thrombin. An ADPase catalyzes the destruction of ADP, decreasing platelet activation.
Thrombopoiesis
Thrombopoietin is a potent megakaryocyte-stimulating factor, acting in synergy with a variety of cytokines. Although these cytokines support several aspects of megakaryocyte development in vitro, genetic elimination (knockout animals) only steel factor (stem cell factor) and thrombopoietin affects megakaryocyte and platelet production in vivo. Thrombopoietin also primes platelets to be more sensitive to platelet agonists. A recombinant form of thrombopoietin is available to increase platelet production in thrombocytopenic patients, although its clinical indications are relatively limited (Figure 10).
REFERENCES