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How Platelets Work: Platelet Function and Dysfunction

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Abstract. **This article briefly reviews (a) how platelets normally function and (b) the clinical approach to disorders of platelet numbers and function.**

Key Words. **platelets, platelet function, platelet function disorders, thrombocytopenia, thrombocytosis, review**

Introduction

Platelets are small cells of great importance in maintaining hemostasis; but they also play a significant role in many pathophysiologic processes, including thrombosis, hemorrhage, inflammation, antimicrobial host defense, and tumor growth and metastasis [1]. Platelets have an increasingly well-defined, critical role in coronary artery disease (the most common cause of human death in developed countries) and in other common diseases including stroke, peripheral vascular disease, diabetes mellitus, and renal glomerular disease [1].

The mammalian platelet is derived from the cytoplasm of megakaryocytes, the only polyploid hematopoietic cell. Polyploid megakaryocytes and their progeny, non-nucleated platelets, are found only in mammals [2]. In all other animal species, cells involved in hemostasis and blood coagulation are nucleated. The elucidation of the evolutionary event or events that resulted in the appearance of mammalian megakaryocytes and platelets, as well as the biological advantage of this system, remains elusive [2].

As shown in Figure 1, megakaryocytes are descended from pluripotent stem cells and undergo multiple DNA replications without cell divisions by the process of endomitosis [3]. In endomitosis, early stages of mitosis occur within the nucleus without formation of the mitotic apparatus and metaphase plate. The nucleus does not divide; and an increase in ploidy is the only sign of endomitosis. Upon completion of endomitosis, polyploid megakaryocytes begin a rapid cytoplasmic expansion phase characterized by the development of an elaborate demarcation membrane system and the accumulation of cytoplasmic proteins and granules essential for platelet function. During the final stages of development (Fig. 2), the megakaryocyte cytoplasm undergoes a massive reorganization into beaded cytoplasmic extensions called proplatelets [3].

Human platelets are 2 to 4 μm in diameter and contain granules (α, dense, lysosomes), mitochondria, and endoplasmic reticulum. Platelets circulate in a concentration of 150 to 400 $\times$ 10^9/L and have a life span of 7 to 10 days.

Platelet Function

Formation of the hemostatic plug at sites of vascular injury begins with the arrest of circulating platelets on exposed collagen and continues with the recruitment of additional platelets into a growing platelet mass that will eventually be stabilized with cross-linked fibrin [4]. Formation of a platelet plug can be thought of as occurring in three phases (Fig. 3): initiation, extension, and perpetuation [4]. *Initiation* occurs when circulating platelets arrest and are activated by exposed collagen and von Willebrand factor (VWF), allowing the accumulation of a platelet monolayer that supports thrombin generation and the formation of platelet aggregates. Key to this phase of platelet activation is the presence of receptors on the platelet surface that can bind to collagen (integrin $\alpha_2\beta_1$ and glycoprotein [GP] VI) and VWF (GPIb-IX-V and integrin $\alpha_{IIb}\beta_3$) and initiate intracellular signaling [4]. *Extension* occurs when additional platelets accumulate on the initial monolayer, a process for which $\alpha_{IIb}\beta_3$ activation is necessary, but not sufficient. Key to this phase is the presence on the platelet surface of receptors that can respond rapidly to locally-generated thrombin, secreted ADP, and released thromboxane A2 (TxA2) to activate

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phospholipase C, increase the cytosolic Ca^{++} concentration, and suppress synthesis of cyclic AMP (cAMP) [4]. Most of the receptors involved in these events are members of the superfamily of G-protein-coupled receptors (Fig. 4). *Perpetuation* refers to the late events of platelet plug formation, when the intense, but short-lived signals arising from G-protein-coupled receptors have faded and the receptors responsible have been desensitized [4]. These late events stabilize the platelet plug and prevent premature disaggregation. Perpetuation is less well understood than initiation and extension; but recent studies point to a central role for outside-in signaling through cell surface integrins and to the signals generated by receptor tyrosine kinases, including members of the Eph and Axl families [4]. Platelets localize, amplify, and sustain the coagulant response at the injury site [5] and release procoagulant platelet-derived microparticles [6].

Table 1. *Characteristic Clinical Features that Differentiate Primary Hemostatic Disorders from Coagulation Disorders*

	Primary hemostatic disorder	Coagulation disorder
Prototypic disorders	Thrombocytopenia platelet function defect von Willebrand disease*	Hemophilia
Bleeding	Immediate	Delayed
Petechiae	Yes	No
Hemarthroses	No	Yes
Intramuscular hematomas	Uncommon	Common
Epistaxes	Common	Uncommon
Menorrhagia	Common	Uncommon

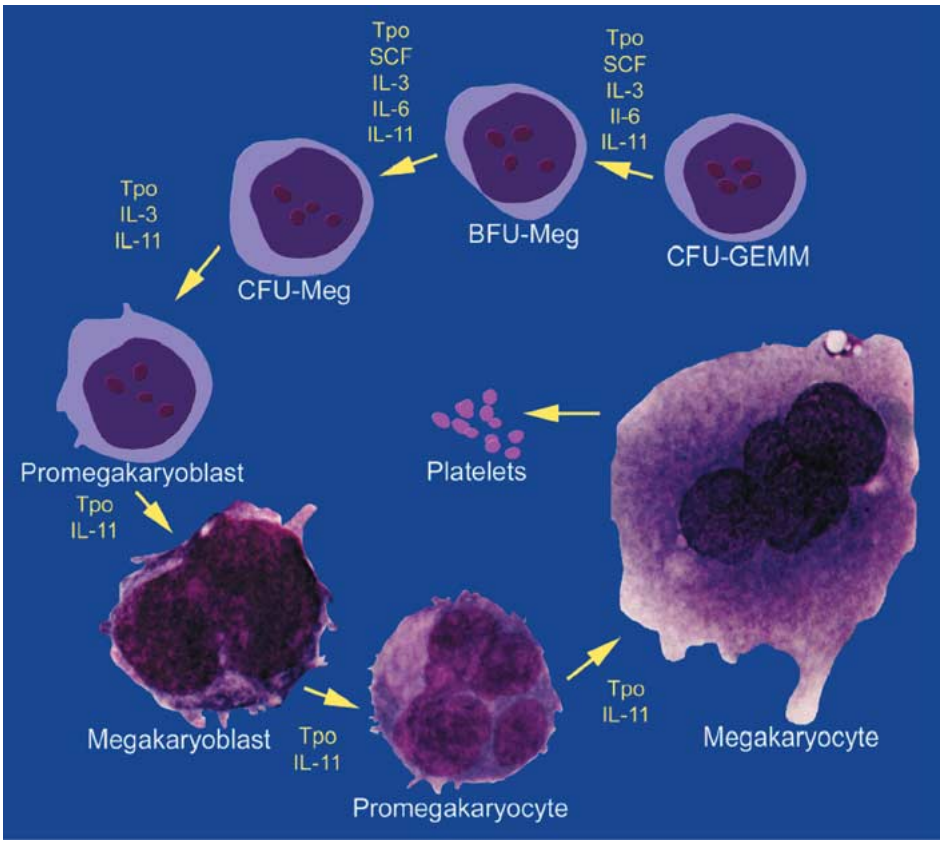
*In the uncommon Type 3 von Willebrand disease, the factor VIII level is low enough for the clinical features to be those of a combined primary hemostatic and coagulation disorder. Reproduced with permission from Michelson AD. The clinical approach to disorders of platelet number and function. In: Michelson AD, ed. *Platelets*. Academic Press/Elsevier Science, 2002.

Platelet Dysfunction

Careful history and physical examination are the keys to differential diagnosis [7]. The characteristic clinical features that differentiate primary hemostatic disorders (thrombocytopenia, platelet function defects, and von Willebrand disease [VWD]) from coagulation disorders (e.g. hemophilia) are listed in Table 1. Three of these clinical features are particularly helpful. First, petechiae are a very strong pointer towards a primary hemostatic disorder and away from a coagulation disorder. Second, hemarthroses are a very strong pointer towards a coagulation disorder and away from

Fig. 1. *Megakaryocyte and platelet development. From a committed myeloid progenitor cell, CFU-GEMM (colony-forming unit–granulocyte-erythroid-macrophage-megakaryocyte), there is evidence for a common intermediate cell (not depicted here) that differentiates into the megakaryocytic, basophilic, and erythroid lineages. The burst forming unit (BFU)-megakaryocyte is committed to megakaryocyte differentiation. Both colony forming unit (CFU)-megakaryocytes and BFU-megakaryocytes express CD34, CD33 and CD41. The CD41 (GPIIb) cell surface antigen is a megakaryocyte lineage marker. The promegakaryoblast is the first morphologically recognizable megakaryocyte precursor in bone marrow. Megakaryoblasts are 15–50 μm in diameter, with large oval nuclei, and a basophilic cytoplasm lacking granules. They have 2 sets of chromosomes (4N). Promegakaryocytes are 20 to 80 μm in diameter and have a polychromatic staining cytoplasm. Megakaryocytes are the largest hematopoietic cells in the bone marrow with diameters up to 150 μm and they have a highly lobulated multilobed nucleus. The cytoplasm stains basophilic. The functions of specific cytokines in megakaryocyte development have been studied in detail. Interleukin (IL)-3, by itself, supports the early stages of megakaryocyte development up to the promegakaryoblast stage before polyploidization. Thrombopoietin (Tpo) is the principal regulator of thrombopoiesis and affects all stages of megakaryocyte development. IL-6, IL-11, and stem cell factor (SCF, kit ligand) also stimulate specific stages of megakaryocyte development, but function only in concert with Tpo or IL-3. Reproduced with permission from Italiano J, Hartwig JH. Megakaryocyte development and platelet formation. In: Michelson AD, ed. Platelets. Academic Press/Elsevier Science, 2002.*

Fig. 2. *Proplatelet model detailing the cytoskeletal mechanics of platelet biogenesis. 1. After commitment to the megakaryocyte lineage, cells undergo endomitosis and cytoplasmic maturation. 2. Platelet production initiates with the formation of large pseudopodia that use unique cortical bundles of microtubules to elongate and form thin proplatelet processes with bulbous ends. 3. These ends contain a peripheral bundle of microtubules that loops upon itself and forms a teardrop-shaped structure. 4. Growth and extension of proplatelet processes is associated with repeated actin-dependent bending and bifurcation, which amplifies free proplatelet ends. Proplatelets form constrictions along their length giving them a beaded appearance. 5. Packets of material destined for assembly into putative platelets move along the proplatelet shaft. It is likely that the linear arrays of microtubules within proplatelets serve as the tracks for the transport of organelles/granules into platelets maturing at proplatelet ends. 6. Proplatelets release from the megakaryocyte body after a rapid retraction, and undergo further fragmentation into individual platelets. (Reproduced from J Cell Biol 1999;147:1310 by copyright permission of The Rockefeller University Press.)*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CFU-GEMM
CFU-Meg
Promegakaryoblast
Platelets
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Fig. 1.

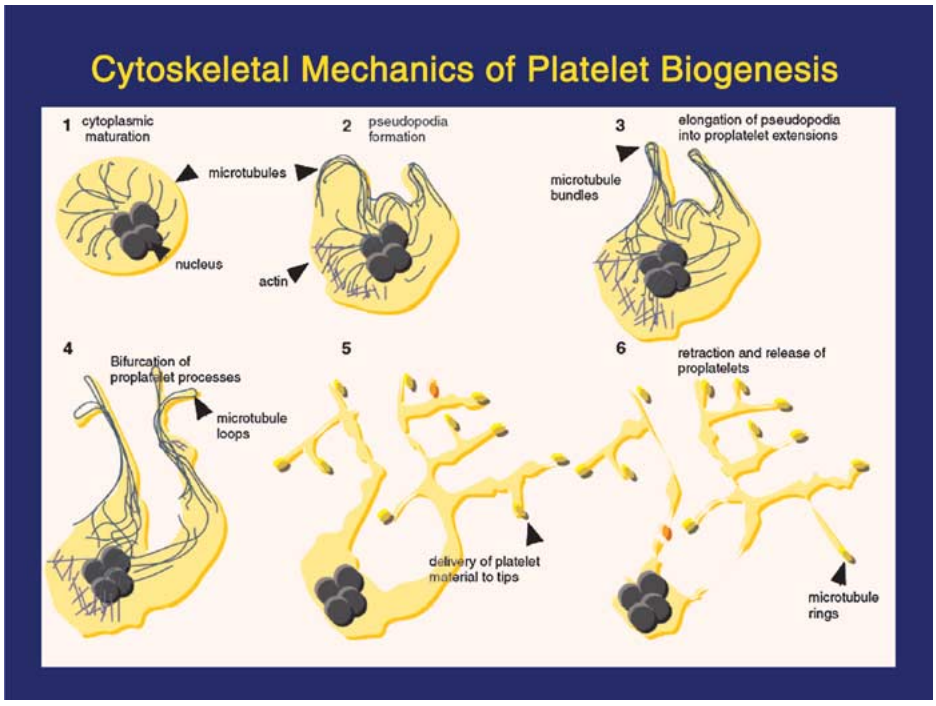
Cytoskeletal Mechanics of Platelet Biogenesis
1 cytoplasmic maturation
microtubules
nucleus
2 pseudopodia formation
actin
3 elongation of pseudopodia into proplatelet extensions
microtubule bundles
4 Bifurcation of proplatelet processes
microtubule loops
5
delivery of platelet material to tips
6 retraction and release of proplatelets
microtubule rings

Fig. 2.

Table 2. *Causes of Thrombocytopenia in the Absence of Leukopenia or Anemia*

Increased platelet destruction	Giant platelet syndromes
Immune thrombocytopenic purpura	Bernard-Soulier syndrome
Disseminated intravascular coagulation	May-Hegglin anomaly
Heparin-induced thrombocytopenia	Fechtner syndrome
Other drug-induced thrombocytopenias	Sebastian syndrome
Systemic lupus erythematosus	Epstein syndrome
HIV-1-related thrombocytopenia	Montreal platelet syndrome
Thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome	Wiskott-Aldrich syndrome
Common variable immunodeficiency	*Sequestration*
Post-transfusion purpura	Hypersplenism
Type 2B von Willebrand disease	Kasabach-Merritt syndrome
Decreased platelet production	*Increased platelet destruction and hemodilution*
Thrombocytopenia with absent radii (TAR) syndrome	Extracorporeal perfusion
Amegakaryocytic thrombocytopenia	

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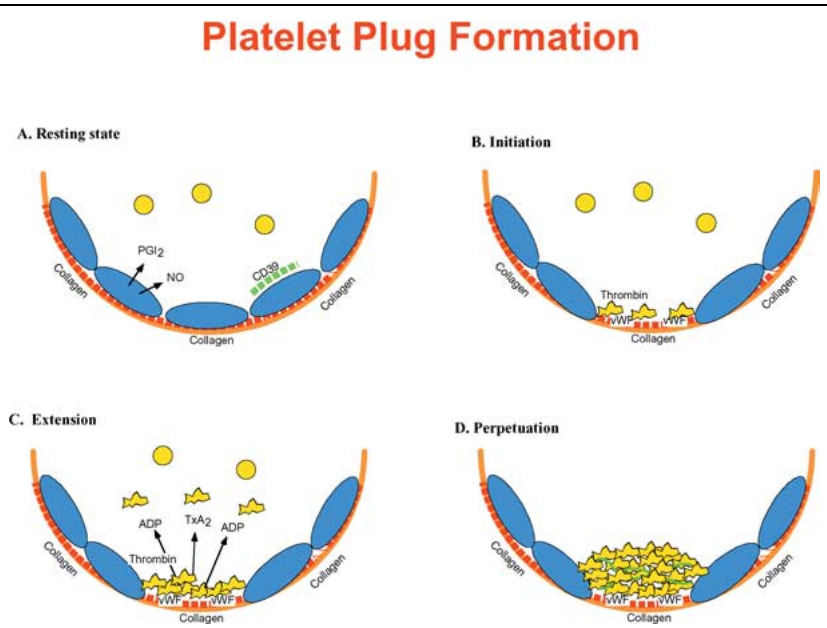

Fig. 3. *Steps in platelet plug formation. (A) Prior to vascular injury, platelets are maintained in the resting state by a combination of inhibitory factors that place a "threshold" that must be surmounted in order for platelets to be activated. These factors include prostaglandin (PG) I_2 and nitric oxide (NO) released from endothelial cells, and CD39, an ADPase on the surface of endothelial cells that hydrolyzes any small amounts of ADP that might otherwise cause inappropriate platelet activation. (B) The development of the platelet plug is initiated by the exposure of collagen and the local generation of thrombin. This causes platelets to adhere via collagen and von Willebrand factor (vWF) and spread on the connective matrix, forming a monolayer. (C) Afterwards, the platelet plug is extended as additional platelets are activated via the release or secretion of thromboxane (Tx) A_2, ADP, and other platelet agonists, most of which are ligands for G protein coupled receptors on the platelet surface. (D) Finally, close contacts between platelets in the growing hemostatic plug, along with a fibrin meshwork, help to perpetuate and stabilize the platelet aggregate. Reproduced with permission from Woulfe D, et al. Signal transduction during the initiation, extension, and perpetuation of platelet plug formation. In: Michelson AD, ed. Platelets. Academic Press/Elsevier Science, 2002.*

Table 3. *Causes of Platelet Function Defects*

Acquired defect

- Uremia
- Myeloproliferative disorders
  - Essential thrombocythemia
  - Polycythemia vera
  - Chronic myeloid leukemia
  - Agnogenic myeloid metaplasia
- Acute leukemias and myelodysplastic syndromes
- Dysproteinemias
- Extracorporeal perfusion
- Acquired von Willebrand disease
- Acquired storage pool deficiency
- Antiplatelet antibodies
- Liver disease
- Drugs and other agents

Inherited

- Platelet adhesion
  - Bernard-Soulier syndrome
  - von Willebrand disease
- Agonist receptors
  - Integrin $\alpha_2ß_1$ (collagen receptor) deficiency
  - GPVI (collagen receptor) deficiency
  - $P2Y_{12}$ (ADP receptor) deficiency
  - Thromboxane A_2 receptor deficiency
- Signaling pathways
  - Gαq deficiency
  - Phospholipase C-$ß_2$ deficiency
  - Cyclooxygenase deficiency
  - Thromboxane synthetase deficiency
  - Lipooxygenase deficiency
  - Defects in calcium mobilization
- Secretion
  - Storage pool disease
  - Hermansky-Pudlak syndrome
  - Chediak-Higashi syndrome
  - Gray platelet syndrome
  - Quebec syndrome
  - Wiskott-Aldrich syndrome
- Aggregation
  - Glanzmann thrombasthenia
  - Congenital afibrinogenemia
- Platelet-coagulant protein interaction
  - Scott syndrome

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a primary hemostatic disorder. Third, in the setting of an injury, immediate excessive bleeding suggests a primary hemostatic disorder (because the initial platelet plug does not form correctly), whereas delayed bleeding suggests a coagulation disorder (because the lack of a well-formed fibrin clot results in gradual breakdown of the initial platelet plug) [7]. Disorders of platelet number and function are listed in Tables 2–4.

Table 4. *Causes of Thrombocytosis*

Primary thrombocytosis

- Essential thrombocythemia
- Chronic myeloproliferative disorders (including polycythemia vera, myelofibrosis with myeloid metaplasia)
- Chronic myeloid leukemia
- Myelodysplastic syndrome

Reactive thrombocytosis

- Infection
- Rebound thrombocytosis (e.g. after chemotherapy or immune thrombocytopenic purpura)
- Tissue damage (e.g. surgery)
- Chronic inflammation
- Malignancy
- Renal disorders
- Hemolytic anemia
- Iron deficiency
- Asplenia (post-splenectomy, post-infarction, or congenital)

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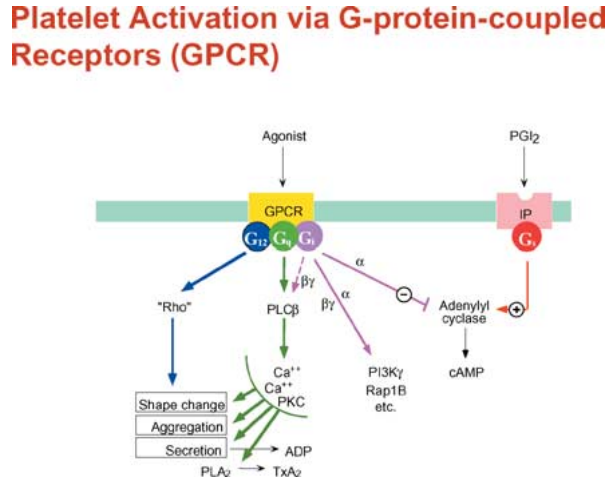

Fig. 4. *Overview of platelet activation via G protein coupled receptors (GPCRs) and G proteins. A number of agonists activate platelets via GPCRs on the platelet surface. Although the details differ from one receptor to the next, critical responses include G_q-mediated activation of phospholipase Cβ isoforms to allow an increase in cytosolic Ca^{++}, activation of phospholipase A_2 and protein kinase C, and G_{12}-mediated guanine nucleotide exchange on Rho family members to support rearrangement of the platelet cytoskeleton (shape change). Activated PGI_2 receptors (IP) stimulate adenylyl cyclase, raising platelet cAMP levels, and causing a generalized inhibition of platelet responses to agonists. G_i family members support the suppression of adenylyl cyclase by platelet agonists and may couple their receptors to other effector pathways as well, including those which activate PI-3-kinaseγ and the Ras family member, Rap1B. Reproduced with permission from Woulfe D, et al. Signal transduction during the initiation, extension, and perpetuation of platelet plug formation. In: Michelson AD, ed. Platelets. Academic Press/Elsevier Science, 2002.*

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