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Platelets, immune-mediated thrombocytopenias, and fetal hemorrhage

Xiaohong Ruby Xu[a,b,c,d], Reid C. Gallant[a,b,c], Heyu Ni[a,b,c,e,f,*]

[a] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada
[b] Toronto Platelet Immunobiology Group, Toronto, ON, Canada
[c] Department of Laboratory Medicine, Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, ON, Canada
[d] The Second Affiliated Hospital, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, P.R. China
[e] Canadian Blood Services, Toronto, ON, Canada
[f] Department of Medicine and Department of Physiology, University of Toronto, Toronto, ON, Canada

KEYWORDS

platelet
thrombosis
immune-mediated thrombocytopenia
integrins
fibrinogen
fibronectin

Abbreviations
von Willebrand factor (VWF)
phosphatidylserine (PS)
idiopathic thrombocytopenic purpura (ITP)
fetal and neonatal alloimmune thrombocytopenia (FNAIT)
post-transfusion purpura (PTP)
reticuloendothelial system (RES)
intracranial hemorrhage (ICH)

ABSTRACT

Platelets are small versatile blood cells generated from megakaryocytes in the bone marrow and cleared in the reticuloendothelial system. Platelet accumulation (adhesion and aggregation) at the site of injury has been considered the first wave of hemostasis. Interestingly, although fibrinogen and von Willebrand factor (VWF) are documented to be essential for hemostasis, fibrinogen/VWF-independent platelet aggregation and thrombosis still occur. Following platelet activation and phosphatidylserine expression, platelets also contribute to cell-based thrombin generation and blood coagulation - the second wave of hemostasis. Most recently, deposition of fibronectin and other plasma proteins onto the injured vessel wall was identified as a "protein wave" of hemostasis, in which platelets may release their granule proteins and thus also contribute to this very early hemostatic event. Due to the central roles of platelets in hemostasis, excessive platelet clearance may lead to bleeding disorders as observed in auto- and alloimmune-mediated thrombocytopenias. In this review, we will introduce several new pathways of thrombosis and hemostasis as well as antibody Fc-independent platelet clearance, which may play an important role in immune-mediated thrombocytopenias. We will also discuss the roles of platelets in fetal hemostasis that may deserve further investigation.

Platelets are versatile cells

Platelets are small anucleate blood cells generated from their precursor megakaryocytes in the bone marrow [1]. After being released into blood, they play key roles in vascular repair and hemostasis. However, inappropriate platelet activation and accumulation may lead to thrombosis, particularly arterial thrombosis [2]. Atherothrombosis (e.g. heart attack and stroke after rupture of an atherosclerotic plaque) is the leading cause of mortality and morbidity worldwide. Recent studies also demonstrate that platelets are versatile and play important roles in inflammation, immune responses, cancer, angiogenesis, lymphatic vessel development, and atherosclerosis etc. [3-5]. The versatility of platelets and the mechanisms of their diverse functions in different environments have emerged as hot topics in the research field.

* Correspondence to: Heyu Ni, MD, PhD, Department of Laboratory Medicine and Pathobiology, Department of Medicine and Department of Physiology, University of Toronto, Scientist of Canadian Blood Services, Platform Director for Hematology, Cancer and Immunological Diseases, St. Michael's Hospital, Room 420, LKSKI - Keenan Research Centre, 209 Victoria Street, Toronto, Ontario, CANADA M5B 1W8. Tel.: +1-416-847-1738.
E-mail address: nih@smh.ca

Platelets in thrombosis and hemostasis - classical and new pathways

At the sites of vascular injury, subendothelial matrix proteins, such as collagen, are exposed to the blood, leading to deposition of von Willebrand factor (VWF) and other plasma proteins, such as fibronectin, onto the vessel wall [6-8]. Platelet VWF receptor GPIbα, by interacting with these anchored VWF molecules, mediates platelet tethering and initiates platelet adhesion. This GPIbα-VWF interaction is essential for initiation of platelet adhesion at high shear stress although it may also contribute to the platelet adhesion at low shear [2,9,10]. Subsequent firm platelet adhesion is mediated by several integrins binding to their ligands on the vessel wall, such as integrin αIIbβ3 (also called GPIIbIIIa) to fibronectin and fibrinogen/fibrin, α5β1 to fibronectin or collagen, and α2β1 to collagen, etc. [11,12]. At low shear (*e.g.* veins) the interactions between platelet integrins and their ligands on the vessel wall may directly initiate platelet adhesion without tethering mediated by GPIbα-VWF [11-13]. Notably, engagement of GPIb and GPVI induces intracellular signalling events that are important for integrin-mediated platelet adhesion [6,12,14]. In addition, locally generated agonists such as thrombin, ADP, and thromboxane A2 also trigger platelets [15,16]. Thus, these signalling events synergistically contribute to platelet adhesion.

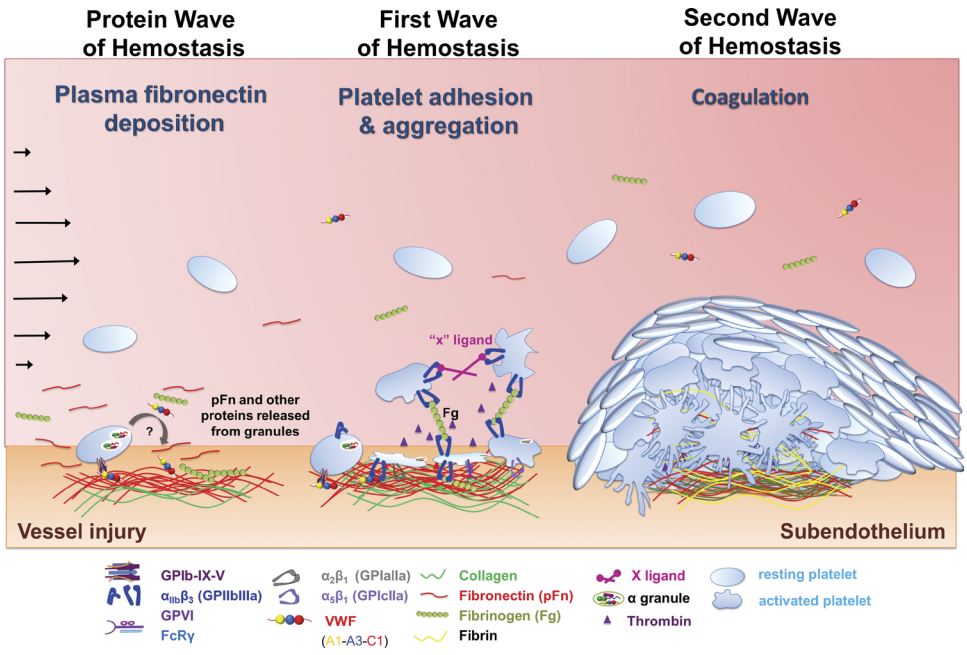

Figure 1. Platelets play key roles in hemostasis and thrombosis. In the event of vascular injury, deposition of plasma fibronectin (pFn) and other proteins onto the vessel wall has been identified as the "protein wave of hemostasis" that occurs prior to platelet accumulation. Platelets may also release their internalized pFn and other adhesive proteins (e.g. fibrinogen and VWF) from their α granules to enhance this very early hemostatic event. The recruitment of platelets to the damaged vessel wall constitutes the first wave of hemostasis. Platelet tethering is initiated by interaction between GPIb-IX-V complex and deposited VWF. Stable adhesion is then mediated by several integrin-ligand interactions (αIIbβ3-fibronectin, fibrin(ogen), VWF; α2β1-collagen; α6β1-laminin; and α5β1-fibronectin etc.) following GPVI-collagen stimulation. Upon platelet adhesion and activation, αIIbβ3 integrin changes its conformation from an inactive, low to high affinity state to bind extracellular ligands, such as soluble fibrinogen, VWF and unidentified X ligands, leading to platelet aggregation and plug formation. Furthermore, platelet activation and phosphatidylserine exposure can promote the cell-based thrombin generation and enhance blood coagulation (the second wave of hemostasis). In a growing hemostatic plug/thrombus, the fibrin and fibrin-fibronectin complex are usually formed at the interface between the injured vessel wall and the platelet plug.

Following the first layer of platelet adhesion and platelet activation, β3 integrins on platelets mediate platelet aggregation by interacting with fibrinogen and other ligand(s). Interestingly, although the requirement of fibrinogen for platelet aggregation has been documented for more than half a century, platelet aggregation and occlusive thrombosis can still occur in mice lacking fibrinogen and/or VWF (i.e. both fibrinogen-dependent and -independent platelet aggregation exist) [17,18]. More strikingly, robust platelet aggregation of these mice can also be easily induced *in vitro* in an aggregometer if non-anticoagulated blood is used [17]. It is currently unclear what these additional β3 integrin ligands are [19,20] and how both fibrinogen-dependent and -independent platelet aggregation pathways may synergistically contribute to thrombosis and hemostasis.

Platelet accumulation (i.e. adhesion and aggregation) at the site of injury has been considered the first wave of hemostasis. The second wave of hemostasis is mediated by the blood coagulation cascade, which can be activated by either the extrinsic (tissue factor) or the intrinsic (contact activation) pathways. Notably, in addition to their key roles in the first wave of hemostasis, platelets also contribute to coagulation. Following platelet activation, platelet surface phosphatidylserine (PS) can harbor the coagulation factors and markedly potentiate thrombin generation (i.e. cell-based thrombin generation) and blood coagulation [21]. In a fibrinogen/VWF-independent platelet aggregation assay, thrombin inhibitors completely blocked ADP-induced platelet aggregation in non-anticoagulated platelet-rich plasma [17], suggesting that even relatively weak signals, such as ADP stimulation, are able to induce or enhance the cell-based thrombin generation. Thus, platelets contribute to both the first and second waves of hemostasis.

Most recently, plasma fibronectin deposition onto injured vessels has been found to be important in hemostasis although it was not reported in the earlier study using plasma fibronectin single deficient mice [7,22,23]. This fibronectin-mediated "protein wave" of hemostasis, which likely involves other plasma proteins, occurs immediately after injury and prior to platelet accumulation (the classic first wave of hemostasis). Although experimental evidence is not yet available, it is conceivable that platelets may release fibronectin and other adhesive proteins from their α granules, which enhances the local plasma fibronectin concentration, facilitating fibronectin deposition and fibronectin self- and non-self-assembly onto the injured vessel wall [7,24]. Interestingly, in afibrinogenemic mice and patients, platelet fibronectin content increases 3-5 fold *via* β3 integrin-mediated plasma fibronectin internalization [24,25]. Releasing these fibronectin molecules from platelets may be an important compensatory mechanism for hemostasis in afibrinogenemic patients. Thus, by releasing α granule proteins (e.g. fibronectin, fibrinogen, and VWF) platelets are also likely involved in the "protein wave" of hemostasis.

Given their important role in the three waves of hemostasis (Figure 1), a dramatic reduction in the number of platelets (thrombocytopenia) may result in significant bleeding as observed in immune-mediated thrombocytopenic disorders [26-28].

Immune-mediated thrombocytopenias

Multiple genetic and environmental factors can affect platelet generation and clearance, including immune-mediated thrombocytopenias [3,26]. Autoimmune thrombocytopenias include idiopathic thrombocytopenic purpura (ITP), drug-induced thrombocytopenias (DIT; e.g. heparin-induced thrombocytopenia, HIT), thrombotic thrombocytopenic purpura (TTP), as well as several secondary thrombocytopenias following autoimmune