Platelets – From Function to Dysfunction in Essential Thrombocythaemia

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Abstract

Platelets are an important component of blood. The main biological role of platelets is to respond to vascular injury and promote thrombus formation to prevent bleeding. However, we now know that platelets also have additional functions in a variety of processes such as immunity, inflammation, coagulation, atherogenesis and tumour metastasis. Platelet disorders commonly lead to defects in haemostasis. Of particular interest is the myeloid proliferative disorder, essential thrombocythaemia (ET). In ET the increased number of platelets leads to an increased risk of blood clot formation and subsequent thrombohaemorrhagic complications. Here we provide a general review of platelet function and activation, as well as more detailed information on the dysfunction of platelets in patients with ET.

Keywords

Haemostasis, megakaryocytes, platelets, platelet adhesion, platelet aggregation, platelet receptors

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Blood platelets could be easily overlooked as they appear inconsequential in blood smears in comparison with red cells and white cells. These small, discoid cells, 1–2µm across,[1] are large in number, with 100–450 billion per litre of healthy blood, and appear as a turbid suspension in platelet-rich plasma (PRP) when erythrocytes/leukocytes are removed by low-speed centrifugation. By 2010, the functional importance of platelets had far surpassed their well-known role in haemostasis and thrombosis, and a recent article[2] highlights many new or suspected roles for platelets in development, as well as in vascular processes such as inflammation, immunity, coagulation, atherogenesis and tumour metastasis. But what are the properties of platelets in healthy individuals, how are the number and function of platelets regulated and how do perturbations due to injury, infection, drugs or acquired or inherited diseases impact platelet function? This article will discuss the biology of platelets and the implications of changes in the normal functioning of platelets with a particular focus on one of the myeloproliferative disorders (MPDs), essential thrombocythaemia (ET).

Platelet Production

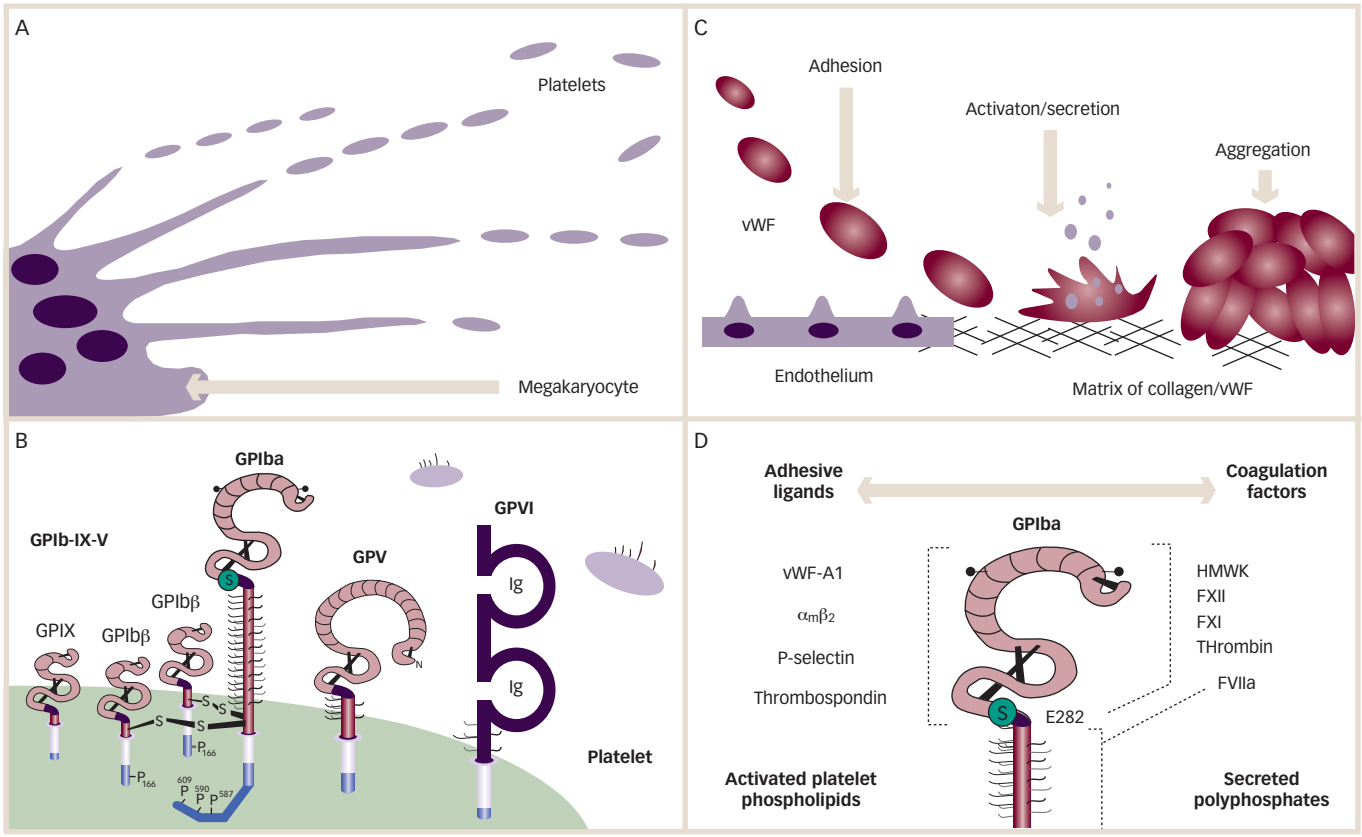
Platelets are derived from the fragmentation of precursor megakaryocytes in the haematopoietic lineage. The mechanisms by which these nucleated cells form elongated structures that break down into individual platelets have been determined in great detail. During maturation over several days, megakaryocytes transform into proplatelets (elongated branched tubular structures containing cytoplasm). New platelets form at the tips of the megakaryocyte protrusions as subcellular granules and organelles are delivered to the proplatelet from the megakaryocyte body (see *Figure 1A*).[3] The mechanism is important, because diseases or drug treatments, particularly chemotherapy, which impair platelet production result in a low platelet count (thrombocytopaenia), which can result in bleeding or deficiency in other platelet functions. A typical adult human might produce a hundred billion (10^{11}) platelets every day, and 5,000–10,000 platelets per megakaryocyte. This can be mimicked *in vitro*, albeit on a far smaller scale, by treating cultured haematopoietic cells with growth factors like thrombopoietin, usually produced by the liver and kidneys.

Platelet Clearance

The balance between platelet production from megakaryocytes and clearance of platelets from the circulation controls the platelet count. There are several causes of platelet clearance.

- Anucleate platelets have a normal lifespan of seven to 10 days, and aged platelets are cleared from the circulation by phagocytosis in the spleen and/or by phagocytic Kupffer cells in the liver.
- There is rapid immunological clearance caused by antiplatelet autoantibodies resulting in autoimmune diseases such as drug-induced autoimmune thrombocytopaenia or idiopathic thrombocytopaenia (ITP).

Figure 1: Platelet Production and Function



Anucleate platelets are released preformed into the bloodstream from tubular structures on megakaryocytes (A). Platelets express unique adhesion receptors such as the glycoprotein (GP) Ib-IX-V complex, which binds von Willebrand factor (vWF), and the collagen receptor, GPVI (B). These receptors support initial platelet adhesion to the injured or diseased vessel wall under shear-flow conditions, leading to rapid platelet activation and secretion, and activation of the integrin $\alpha_{IIb}\beta_3$, which binds vWF or fibrinogen and mediates platelet aggregation (C). Concomitant with thrombus formation, platelets play an important role in coagulation through receptors such as GPIbα (the ligand-binding subunit of GPIb-IX-V), which binds adhesive ligands, and coagulation factors and thrombin by expression of phospholipids on activated platelets involved in assembly of procoagulant complexes, and/or by secretion of procoagulant factors such as polyphosphates from dense granules that potentially activate coagulation factors of the intrinsic pathway (convert factor XII [FXII] to FXIIa).

- Refrigeration of platelets used for transfusion (<15ºC, one hour) results in accelerated clearance upon reperfusion, with an apparent role for platelet glycoprotein (GP) Ibα and $\alpha_M\beta_2$ on Kupffer cells.[4]
- Platelet 'apoptosis' induced by drugs or chemicals, for example chemotherapeutics (either *in vivo* or *in vitro* treatment prior to reperfusion), can result in increased apoptotic markers and rapid clearance of systemic platelets, as well as potential effects on megakaryocytes and platelet production, resulting in thrombocytopaenia.

In the case of cold-related platelet lesions, alterations of platelet receptor glycosylation and clustering of receptors on the surface may trigger clearance. It is also generally recognised that platelet receptor surface expression levels decrease with age, and metalloproteinase-mediated ectodomain shedding potentially plays some part in regulating platelet clearance. In this regard, there is experimental evidence that sequestering of 14-3-3ζ or other proteins by the cytoplasmic domain of GPIb-IX-V, a platelet adhesion receptor, could control 14-3-3ζ-dependent regulation of interactions between cellular death/survival factors.

Platelet Adhesion Receptors

Platelets express unique receptors adapted to fulfil their role and enable rapid transition from a resting circulating state to an adherent activated state under high shear rates (see *Figure 1B* and *1C*). GPIb-IX-V is constitutively expressed on the surface of resting platelets, but the binding site for its major adhesive ligand, von Willebrand factor (vWF), is non-functional, preventing interaction with plasma vWF. It is only when the vWF-binding domain of GPIbα (N-terminal 282 residues) or the GPIbα-binding domain of vWF (A1 domain) is activated that the adhesive interaction occurs.[5] The GPIbα–vWF-A1 interaction is induced *in vivo* by high shear stress resulting in an active conformation of GPIbα, vWF or both, or by immobilisation of vWF in the subendothelial matrix. This allows GPIb-dependent platelet adhesion and activation at sites of vascular injury (haemostasis) or disease (atherothrombosis or vascular stenosis generating pathological shear stress). Functional studies, molecular evidence and *in silico* modelling reveal how shear-induced conformational changes enhance the bond strength for the interaction through 'catch–slip' bonds as the shear rate increases. This molecular adaptation enables GPIb/vWF-dependent platelet adhesion under pathological shear conditions.[6–9]

GPIb-IX-V comprises multiple subunits, all of which are members of the leucine-rich repeat family of proteins (see *Figure 1B*). GPIbα (~135kDa) is disulphide-linked to two GPIbβ subunits (~25kDa), and non-covalently associated with GPIX (~20kDa) and GPV (~82kDa). The ligand-binding domain of GPIbα is elevated from the membrane by a sialomucin core, and interacts with multiple adhesive ligands such as vWF and thrombospondin, receptors such as P-selectin (activated platelets or endothelial cells) or $\alpha_M\beta2$ (activated leukocytes) and coagulation factors such as factor XII (FXII), FXI, thrombin and high-molecular-weight kininogen; this receptor clearly plays a central role in

platelet function.[5] Proteolytic removal of GPV by thrombin or other proteinases facilitates thrombin-induced signalling. The intracellular domain of GPIb-IX-V, comprising the cytoplasmic tails of GPIbα, GPIbβ, GPIX and GPV, is linked to structural (filamin-A, or actin-binding protein) or signalling adaptor proteins (calmodulin, 14-3-3ζ, p85), which enables extracellular ligand binding to initiate intracellular signalling pathways, resulting in platelet activation, cytoskeletal rearrangements and integrin activation. Congenital deficiency of GPIb-IX-V, Bernard–Soulier syndrome, arises from many different individual defects and is associated with giant platelets and thrombocytopaenia, as well as impaired platelet response to vWF, thrombin and other agonists.[10]

The GPIbα–vWF-A1 interaction can be induced *in vitro* by applying pathological shear stress through the use of a cone-plate viscometer, by immobilising vWF on plastic or glass in flow chambers or static adhesion assays, or by using artificial vWF activators such as ristocetin (a bacterial antibiotic) or botrocetin (a snake toxin) that allow plasma vWF to bind platelets, which forms the basis of standard aggregation assays. Importantly, gain-of-function mutations within GPIbα (platelet-type von Willebrand's disease) or vWF-A1 (type 2b von Willebrand's disease) can enable platelet GPIbα to bind plasma vWF *in vivo* or *in vitro*.

GPVI is a platelet-specific receptor consisting of two extracellular immunoglobulin domains (see *Figure 1B*) and is non-covalently associated with the Fc receptor γ-chain, FcRγ, required for GPVI surface expression and transmitting signals upon cross-linking of GPVI by ligands.[11,12] GPVI binds collagen and laminin.[13] Non-physiological ligands include a cross-linked collagen-related peptide and a number of snake toxins (convulxin, alborhagin and others), which can be used to assess GPVI-dependent platelet function *in vitro*. The cytoplasmic domain of GPVI binds the signalling molecule, Lyn, and calmodulin.[11] GPVI and GPIbα are physically and functionally co-associated on the platelet surface, providing a potent recognition–signalling complex for vWF/collagen.[14] These receptors therefore play an important role in the initiation of atherothrombosis at high shear stress and where there is exposed collagen.

Human defects of GPVI may be either an acquired deficiency, resulting from anti-GPVI autoantibodies or other causes, or a congenital deficiency where GPVI is not expressed or is expressed in a dysfunctional form with defective signalling to $\alpha_{IIb}\beta_3$.[15] Of 13 reported cases of GPVI defects, 12 involve females and commonly there is an associated immune dysfunction. Platelets in these patients typically show defective aggregation to collagen or other GPVI ligands but aggregate in response to other platelet agonists.

On platelet activation, P-selectin and CD40 ligand are rapidly translocated to the platelet surface and are subsequently cleaved to generate soluble forms that are biologically active.[16] The soluble forms, soluble P-selectin (sP-selectin) and soluble CD40 ligand (sCD40L), promote coagulation by inducing tissue factor (TF) expression on monocytes and endothelial cells.[17] Soluble CD40L also causes platelet activation and appears to be required for thrombus formation *in vivo*.[18]

Platelets – Role in Haemostasis and Thrombosis

The main role of platelets in thrombus formation is to recognise a vascular site of injury, rapidly adhere, become activated, spread over the surface and recruit additional platelets to form an aggregate or thrombus (see *Figure 1C*). This serves at least two purposes: to prevent blood loss and facilitate wound healing by forming a plug and providing a pro-coagulant surface to accelerate the coagulation cascade; and to fight infection by rapid secretion of bioactive substances from granules that can activate immune cells. By providing an adhesive surface on the thrombus mass that allows direct interaction with leukocytes, these can then migrate to sites of infection or disease. In this regard, a co-ordinated response involves specific platelet surface receptors, rapid agonist-induced secretion of prothrombotic, procoagulant and proinflammatory factors, cytoskeletal changes associated with cell spreading and migration and altered membrane properties promoting coagulation, which all combine to progress from initial injury to arrest of bleeding.

The link between haemostasis and thrombosis is an important one. In the case of injury or infection, a rapid platelet response to form a thrombus is vital to prevent blood loss and promote healing. However, if the same sequence of events occurs in a diseased or damaged blood vessel, for example where an atherosclerotic plaque ruptures, then the resulting thrombosis can block blood supply to the heart or brain and cause heart attack or stroke. Similarly, platelet response to exposed collagen in an arthritic joint can promote unwanted inflammation, causing disease.[19] The key to therapeutic management of thrombosis, therefore, is to inhibit pathological thrombosis but without inhibiting normal haemostasis.[20,21] Existing antiplatelet drugs such as aspirin or clopidogrel (one of the world's biggest-selling medicines), which are used in the treatment or prophylaxis of thrombotic disease, can pose an unacceptable bleeding risk. The most recent approach to overcoming this problem is to consider the role of shear stress in thrombosis and haemostasis (haemorheology).[22,23] In the healthy circulation, fluid shear rates in veins or arteries/arterioles might vary from ~100s^{-1} to ~1,800s^{-1}.[24] However, in a stenotic blood vessel or sclerotic large artery, turbulent shear rates can exceed 10,000s^{-1} (or even higher than this). This profoundly affects the capacity of platelets to form a thrombus, and a select group of platelet receptors and other clotting pathways come into play.[7,25] In experimental models of thrombosis, new ways of targeting thrombosis under pathological shear conditions, without affecting bleeding times, have raised the possibility of safer antiplatelet drugs in the future.[26,27]

The basic steps involved in the transition of circulating resting platelets in the bloodstream to formation of a blood clot or thrombus can be briefly summarised as follows (see *Figure 1C*).

- Activation of platelets in response to signal transduction after engagement of GPIb-IX-V or GPVI induces shape change, cytoskeletal rearrangement, secretion of granule contents such as adenosine diphosphate (ADP) or thromboxane A2 (TXA2), surface expression of P-selectin on α-granules and 'inside-out' activation of the integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa), and other integrins.
- Aggregation is mediated by activated $\alpha_{IIb}\beta_3$ binding to vWF or fibrinogen, and potentiated at high shear by ADP acting at G protein-coupled purinergic receptors, P2Y1 and P2Y12, as well as by other platelet receptors such as C-type lectin-like receptor 2 (CLEC-2), CD40L, and semaphorin-4D, which activate platelets within a developing thrombus.
- Activated platelets greatly accelerate the coagulation cascade, generating active thrombin, which can further activate platelets through the G protein-coupled thrombin receptors, protease-activated receptor 1 (PAR-1) and PAR-4, which stabilises the thrombus by formation of fibrin. This is followed by $\alpha_{IIb}\beta_3$-mediated

clot contraction involving contraction of the cytoskeletal actin filaments in platelets.
- The progression and development, or embolisation, of a developing thrombus could be controlled in part by negative regulation by metalloproteinase-mediated ectodomain shedding of platelet receptors, such as GPIbα (ligand-binding subunit) of GPIb-IX-V or GPVI, or by the switching on of inhibitory signalling pathways downstream of receptors such as leukocyte-associated immunoglobulin (Ig)-like receptor 1 (LAIR-1) or platelet endothelial cell adhesion molecule 1 (PECAM-1).

This sequence of events could be envisaged where there is damage of the blood vessel wall leading to exposure of collagen/vWF in the subendothelial matrix; however, other physiological or pathological circumstances are also capable of initiating thrombus formation. An important example is found in some forms of autoimmune disease, where antibodies activating platelets via the Fc receptor, FcγRIIa, can trigger thrombosis. Another example is coagulopathy caused by aberrant generation of thrombin, a potent platelet agonist, acting at PAR-1 and PAR-4.

Platelet Interaction with Leukocytes and the Endothelium

The interaction between platelets, leukocytes and the endothelium can occur in different ways. Platelets can first form conjugates with leukocytes and support leukocyte recruitment to the endothelium via activation of leukocyte adhesion receptors. Alternatively, platelets adherent on the endothelium can chemoattract leukocytes and provide a sticky surface for neutrophil–endothelium interaction. The net result of these events is the infiltration of inflammatory cells into the vessel wall.[28,29] Platelets therefore play additional roles beyond haemostasis and thrombosis. Platelet-mediated inflammation provides the basis for plaque formation before actual vessel occlusion; platelets thus link the diverse processes that culminate in atherogenesis.

Secretion of Growth Factors from Platelets

Activated platelets rapidly release a multitude of growth factors from intracellular storage granules. These growth factors include platelet-derived growth factor (PDGF), a potent chemotactic agent, and transforming growth factor-β (TGF-β), which stimulates the deposition of extracellular matrix (ECM) as well as performing other functions. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other growth factors produced by platelets include basic fibroblast growth factor (bFGF), insulin-like growth factor 1 (IGF-1), platelet-derived epidermal growth factor (PDEGF) and vascular endothelial growth factor (VEGF). These factors promote inflammation by stimulation of leukocytes as part of the normal defence against infection, as well as pathologically in inflammatory disease.

The intracellular granule-expressed protein P-selectin mediates adhesion between activated platelets and neutrophils via the counter-receptor, P-selectin glycoprotein ligand-1 (PSGL-1). Platelets also secrete platelet agonists, such as ADP or TXA2, that act in an autocrine or paracrine fashion as prothrombotic factors, and adhesive proteins such as vWF or thrombospondin, which promote platelet adhesion under shear stress by binding to GPIbα or other receptors.[21]

Other important secreted factors, including coagulation factors and fibrinolytic proteins, regulate coagulation. Polyphosphate, a secreted procoagulant molecule, is a highly negatively charged multimer that appears to play a key role in activation of the intrinsic coagulation pathway by activation of FXII.[30] In experimental models, FXII/FXI-initiated coagulation is important in formation of a stable occlusive thrombus, although the intrinsic pathway seems dispensable for normal haemostasis.[31] The receptor GC1qR/p33 is also expressed on activated platelets,[32] and can assemble a procoagulant complex involving FXII, high-molecular-weight kininogen and pre-kallikrein that is involved in the initiation of the intrinsic coagulation pathway.

Together, the capacity of circulating platelets to transport diverse prothrombotic and procoagulant substances throughout the bloodstream, and to rapidly release them into the local environment when triggered, has significant consequences in health and disease.

Cross-talk Between Platelets and the Coagulation Cascade

Platelet activation and activation of the coagulation cascade are complementary processes. Coagulation factors bind to platelets through either glycoprotein receptors or through anionic phospholipids exposed on the outer surface of the plasma membrane after platelet activation (see *Figure 1D*). For example, binding of collagen to GPVI activates platelets, exposes phosphatidylserine and supports thrombin formation and clot stabilisation.[33] Collagen–GPVI interaction is also responsible for the shedding of membrane blebs into the circulation, which provides procoagulant microvesicles. Bleb formation and phosphatidylserine exposure rely on the induction of prolonged increases in intracellular calcium resulting from platelet activation by ADP, TXA2, thrombin and collagen. ADP is also responsible for the induction of platelet procoagulant activity through interaction with P2Y1 and P2Y12.[34] Platelet secretion products contribute to the procoagulant activity of activated platelets by providing factor V, factor VIII and fibrinogen. Activated platelets support the initiation phase of coagulation by providing binding sites for FXI and prothrombin. These functions reveal the dual role of platelets in thrombus formation and coagulation (see Figure *1D*). Thus, sP-selectin might be involved in phosphatidylserine exposure in monocytes and in the genesis of leukocyte-derived microparticles containing active TF, which enhance thrombin generation and fibrin deposition through a PSGL-1-dependent mechanism.[35]

In addition, sCD40L induces platelet P-selectin expression, aggregation, leukocyte activation, platelet–leukocyte conjugation and platelet release of reactive oxygen intermediates.[36]

Role of Platelets in Immunity

Platelets also appear to play an active role in both innate and adaptive immunity.[37,38] For example, platelet adhesive interactions, such as GPIbα with endothelial cells (P-selectin) or leukocytes ($\alpha_M\beta_2$), or activated platelet P-selectin with leukocytes (PSGL-1), can support leukocyte rolling and activation on a mural thrombus. In addition, the secretion of cytokines can recruit leukocytes to sites of tissue damage. Platelets also express immune receptors such as FcγRIIa,[39] and immunologically relevant molecules such as CD40L and toll-like receptors, which functionally modulate innate immunity. Platelets also interact with Gram-positive or -negative bacteria and spirochetes, which can activate platelets and promote an inflammatory or immune response through secreted proinflammatory factors. Bacteria are also a potentially important risk factor for cardiovascular thrombotic disease.[39–41]

Abnormal Functioning of Platelets

It is clear that platelets play a major physiological role that can be either beneficial or deleterious depending on the circumstances. Platelet disorders lead to defects in primary haemostasis and have signs and symptoms different from coagulation factor deficiencies (disorders of secondary haemostasis). An abnormality or disease of platelets is called a thrombocytopathy and can take differing forms: e.g. low platelet number, increased platelet number or loss of function. Low platelet number (thrombocytopaenia), characterised by a platelet count of <100–150 x 10^9 per litre of blood, can cause excessive bleeding and may be drug induced, e.g. heparin-induced thrombocytopaenia,[42] or of immune origin, e.g. ITP. ITPs are acquired autoimmune disorders mediated by the production of antiplatelet antibodies, which are commonly directed against GPIIb/IIIa and GPIb/IX on the platelet surface.[43,44] Platelets may be affected by a decrease or loss of function (thrombasthaenia) such as in Glanzmann's thrombasthenia. Platelets in Glanzmann's thrombasthenia lack GPIIb/IIIa due to either an inherited mutation or acquired immune disorder.[45] ET is an example of a disease in which there is an increased number of platelets that leads to an increased risk of blood clot formation.[46,47]

Platelets and Essential Thrombocythaemia

ET is a myeloid proliferative disorder characterised by an increase in the peripheral blood platelet count that is associated with bone marrow megakaryocyte hyperplasia, without associated erythrocytosis or leukoerythroblastosis.[47] ET manifests clinically as thrombocytosis[46] and is associated with a broad spectrum of microvascular disturbances[48] and relatively frequent thrombohaemorrhagic complications.[49] In ET, platelets are larger, less mature and more responsive to activation compared with normal platelets. These abnormal platelets are present in a range of states including resting, activated and desensitised. Biochemically, the most frequently detected abnormality is impaired epinephrine-induced platelet aggregation,[50] caused by loss of α2-adrenergic receptors.[51] In addition, defects in arachidonic acid metabolism,[52] an acquired storage pool defect of dense granules or abnormalities of platelet receptors such as GPIIb/IIIa[53] affect platelet aggregation in ET.

Essential Thrombocythaemia Clinical Expression

The contribution of platelets to thrombotic risk is supported by several lines of evidence. First, histological studies of erythromelalgia demonstrate platelet-rich arteriolar microthrombi rich in vWF and minimal fibrin.[54] Second, in most patients with ET, erythromelalgia is particularly sensitive to aspirin. The contribution of platelet count as a risk factor is presumed from clinical data showing that cytoreductive therapy reduces the incidence of thrombosis.[55] Finally, a recently published analysis of the European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) data concluded that antiplatelet therapy was significantly associated with a lower risk of cardiovascular events, but that there was no clear relationship between such events and phlebotomy or cytoreductive therapy.[56] Although these data implicate the platelet in the pathogenesis of these events, any extrapolation of the role of cytoreductive therapy or phlebotomy should be interpreted with caution.

Published studies on ET pregnancies report live birth rates of 50–70% and spontaneous abortion rates of 25–50%.[57,58] In a recent report of 103 pregnancies that occurred in 62 women with ET, about 50% of first pregnancies had complications, although no case of acute coronary syndrome or myocardial infarction was reported during pregnancy or post-partum.[59] Although a decrease in platelet count during pregnancy is well documented, pregnancies in ET patients frequently end in early spontaneous abortions during the first trimester.[60] Their occurrence cannot be predicted from the disease course, platelet count or a specific therapy.

Thrombosis and Haemorrhage in Essential Thrombocythaemia

Approximately 20% of ET patients will have had a major thrombotic event at the time of diagnosis and approximately another 20% will have an event subsequently. This makes venous and, more commonly, arterial thrombosis the leading causes of morbidity and mortality in ET. However, the precise incidence of thrombosis in ET is hard to ascertain. Confounding factors include the retrospective nature of the literature, comprising relatively small uncontrolled case series, variable event definition and patient selection and reporting bias. Established risk factors for thrombosis in ET are older age (over 60 years) and previous thrombotic events. Recently, leukocytosis has also been identified as an additional risk factor.[61] Numerous mechanisms, including blood hyperviscosity and quantitative/qualitative abnormalities of blood cells, have been postulated to be at the origin of the hypercoagulable state in these patients.[62] The increased thrombotic state in ET patients is still not completely understood but it is thought that increased platelet number, abnormal platelet function, platelet and leukocyte activation, platelet–leukocyte aggregation and endothelial activation may all be contributing factors.

A higher platelet TF expression has been shown in ET patients when compared with controls not age- and sex-matched.[63] In addition, elevated plasma levels of vWF:antigen (Ag), a large glycoprotein mainly synthesised by the endothelial cells that plays an important role in platelet thrombus formation,[64] have been observed in ET patients, particularly those with previous thrombosis. Increased factor V levels are also associated with increased thrombotic risk in ET. Presumably, increased levels of these factors result in the acceleration of clotting, leading to enhanced risk of thrombus formation. The higher thrombin generation values observed in ET and polycythaemia vera patients with previous thrombosis are in agreement with these findings.[65] It has been shown that the presence of acquired activated protein C resistance (aAPCR) phenotype and elevated levels of coagulation factors are associated with increased risk of thrombosis.[66] ET patients with thrombosis have been shown to have significantly higher values than patients without thrombosis for a number of factors including: reticulated platelet (RP) percentage, aAPCR, levels of factors V and VIII, vWF:Ag, sP-selectin and sCD40L.[67] In a multivariate analysis, RP percentage, factor V levels and aAPCR were independently associated with an increased risk of thrombosis. The mechanisms causing aAPCR are not clear, although it has been suggested to be related to antiphospholipid antibodies, reduced P-selectin levels and high levels of factors V and VIII and vWF:Ag.[68]

Platelet Activation in Essential Thrombocythaemia

Enhanced platelet activation in ET was initially documented over a decade ago. Activated platelets interact with other blood components (both cellular and circulating) and have the capacity to provoke endothelial activation/damage. Further features of platelet activation include the formation of platelet microparticles, which are associated with the expression of platelet procoagulant activity. These microparticles can be increased in ET and are correlated with

thrombosis (see also section below).[69] Increased expression of P-selectin, thrombospondin and the activated fibrinogen receptor GPIIb/IIIa, have also been demonstrated in ET and show variable correlation with thrombosis.

Currently, the exact pathogenesis of platelet activation in ET, and the other MPDs, is unknown. A large proportion of patients have a deficiency of lipoxygenase, which could increase the availability of endoperoxides to produce TXA2.[70] However, the same patients have a tendency for haemorrhagic rather than thrombotic diathesis. Alternative explanations for increased platelet activation include an effect of the janus kinase 2 (JAK2)-activating mutation (found in approximately half of patients with ET), interaction of abnormal haematocrit, activated white cells, turbulent flow or an increase in the known priming effect of thrombopoietin due to elevated thrombopoietin levels.[71] There is also a suggestion that JAK2 affects cMPL cell surface localisation and stability, which may have implications for the pathogenesis of platelet activation.[72]

Platelet Microparticles in Essential Thrombocythaemia

Like patients with other thromboembolic diseases, ET patients show higher levels of platelet-derived microparticles than healthy subjects. However, this is not necessarily a consequence of increased platelet number in ET because microparticle numbers do not correlate with platelet numbers in either ET patients or controls. This suggests that microparticle formation may be a regulated rather than a constitutive process. Despite the large proportion of platelet-derived microparticles in ET patients, the actual number of these microparticles with markers of platelet activation (CD62P and CD63) is not increased.[73] This could be explained by most ET patients receiving antithrombotic drugs at the time of blood sampling, which may affect platelet activation. For example, aspirin inhibits the expression of CD62P and CD63 on platelets.

However, patients with ET can show an increase in CD62E-positive microparticles.[74] The presence of CD62E-positive microparticles suggests endothelial activation, a finding substantiated by the higher concentrations of mature vWF in ET. Since the increased mature vWF concentration is not accompanied by a rise in propeptide levels, it is believed that this is a chronic, rather than acute, endothelial activation. An explanation for this could be an interaction between platelets (or platelet fragments) and endothelial cells resulting in cellular activation and generation of microparticles of bilineage origin. Increased numbers of CD41/CD62E-positive microparticles may be of pathophysiological significance since they appear to be related to risk factors for thrombosis in ET.

Summary

Platelets have multiple and complex physiological functions, most notably their pivotal role in thrombosis and haemostasis. Our increasing knowledge of the biological role of platelets has allowed us to more clearly appreciate the physiological relationships they can have with thrombi and leukocytes. Such information gives us greater insight into the deleterious effects of platelets in diseases such as ET. Further improving our understanding of the function and dysfunction of platelets will allow us to develop appropriate pharmacotherapeutic interventions to maintain platelet activity from both a functional and quantitative perspective. ■

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