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Cells in focus

Platelets: Pleiotropic roles in atherogenesis and atherothrombosis

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ABSTRACT

Platelets are small, anucleate blood elements of critical importance in cardiovascular disease. The ability of platelets to activate and aggregate to form blood clots in response to endothelial injury, such as plaque rupture, is well established. These cells are therefore important contributors to ischaemia in atherothrombosis, and antiplatelet therapy is effective for this reason. However, growing evidence suggests that platelets are also important mediators of inflammation and play a central role in atherogenesis itself. Interactions between activated platelets, leukocytes and endothelial cells trigger autocrine and paracrine activation signals, resulting in leukocyte recruitment at and into the vascular wall. Direct physical interaction may contribute also, through platelet adhesion molecules assisting localization of monocytes to the site of atherogenesis and platelet granule release contributing to the chronic inflammatory milieu which leads to foam cell development and accelerated atherogenesis. Recent studies have shown that antiplatelet therapy in animal models of accelerated atherogenesis can lead to decreased plaque size and improve plaque stability. This review examines the complexity of platelet function and the nature of interactions between activated platelets, leukocytes and endothelial cells. We focus on the growing body of evidence that platelets play a critical role in atherogenesis and contribute to progression of atherosclerosis.

Cell facts

- Platelets activation and aggregation is responsible for blood clots after plaque rupture, leading to ischaemic heart disease.
- Platelet activation also plays an important role in inflammation.
- Growing evidence suggests platelets are an important contributor to accelerated atherosclerosis.

1. Introduction

Platelets are small cell fragments of large importance in medicine. They are involved in many physiological processes, particularly haemostasis through their ability to aggregate and form clots in response to activation. Therefore platelet dysfunction is most often associated with disorders of bleeding and thrombosis (Linden et al., 2004). However, platelets also play an important role in inflammation and can influence the phenotype of other blood and vascular cells through cell–cell signalling (Linden and Furman, 2005; Barnard et al., 2005). For this reason platelets contribute to many other non-haemostatic disorders, from cystic fibrosis and arthritis to diabetes and cancer (O'Sullivan et al., 2005; Linden et al., 2004; Boilard et al., 2010). In this review we focus on the pleiotropic roles platelets play in atherothrombosis and review the growing evidence linking platelet activation to accelerated atherogenesis.

Platelets circulate in blood as small, granular, anuclear discs. These 3.0 by 0.5 μm elements circulate in laminar blood flow near the apical surface of the endothelium. Upon activation they undergo rapid metamorphic changes to spread and adhere to damaged endothelial surfaces, release granules, aggregate with other platelets and interact with leukocytes (Hartwig, 2002).

Platelets are highly granular. Alpha granules contain a variety of adhesion molecules, chemokines, coagulation and fibrinolysis proteins, growth factors, immunologic molecules and other proteins (Table 1). Dense granules contain ionic calcium, magnesium, phosphate and pyrophosphate as well as ATP, GTP, ADP and GDP nucleotides and the transmitter serotonin. Granule secretion therefore deposits thromboinflammatory mediators at the site of platelet activation, and results in expression of adhesion molecules not normally expressed on the platelet surface to facil-

Abbreviations: ADP, adenosine diphosphate; GP, glycoprotein; PKC, protein kinase C; PMP, platelet derived microparticles; PSGL-1, P-selectin glycoprotein ligand 1; vWF, von Willebrand factor.

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Table 1
Components of platelet alpha granules.

Adhesion molecules	Fibronectin, fibrinogen, integrin αIIbβ3, integrin $\alpha_v\beta_3$, P-selectin, vWF, PECAM-1, CD9, GPIb-IX-V complex.
Chemokines	B-thromboglobulin, growth-regulated oncogene α, interleukin 8, macrophage inflammatory protein 1α, monocyte chemotactic protein 3, neutrophil activating protein, platelet factor 4, RANTES.
Coagulation proteins	Factor V, factor VIII, high molecular weight kininogen, multimerin.
Fibrinolysis proteins	α2-Macroglobulin, plasminogen, plasminogen activator inhibitor 1.
Growth factors	Basic fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, insulin-like growth factor 1, platelet derived growth factor, transforming growth factor β, vascular endothelial growth factor.
Immunologic molecules	B1H globulin, c1 inhibitor, factor D, IgG.
Other proteins	α1 antitrypsin, albumin, osteonectin.

itate platelet adhesion/aggregation. By changing the expression and activation status of these surface molecules, platelets are able to rapidly and markedly change their phenotype. This ability to rapidly recognise, adhere to and release thromboinflammatory mediators at specific sites of vascular injury are important to the role platelets play in both acute atherothrombosis and in chronic atherogenesis.

2. Cell origin and plasticity

Platelets are sub cellular fragments released from megakaryocytes, which are rare myeloid cells found largely in the bone marrow and lung (Ogawa, 1993). Once released by megakaryocytes, platelets circulate in the blood for about 7–10 days after which they are sequestered and phagocytosed in the spleen and liver. Platelet activation may shorten the circulating life of platelets via aggregation, early sequestration or formation of platelet derived microparticles (PMPs).

Signal transduction resulting from ligands binding receptors on the surface of platelets leads to elevation of intracellular calcium and activation of several enzymes, such as calpain and protein kinase C (PKC) (Woulfe et al., 2002). This facilitates PMP production by degrading structural proteins including actin-binding protein, talin and the heavy chain of myosin (Nieuwland and Sturk, 2002). Concurrently, the platelet cell membrane loses its organized asymmetrical distribution and negatively charged aminophospholipids phosphatidylserine and phosphotidylethanolamine become expressed on the surface. Expression of these aminophospholipids facilitates interaction with the coagulation system, serving as the site of the thrombin production. These procoagulant phospholipids are therefore also expressed on PMPs which bud off from the platelet, forming procoagulant microparticles (Nieuwland and Sturk, 2002). Furthermore, platelets and PMPs share glycoprotein receptors such as GPIb, PECAM-1, and integrin αIIbβ3. Subpopulations may also express P-selectin from platelet granules, suggesting PMPs can participate in cellular interactions, adhesion and aggregation (Woulfe et al., 2002; Reed, 2002; Nieuwland and Sturk, 2002).

In addition to being formed as a result of agonist-induced platelet activation, PMPs may form as a result of complement activation/damage to platelets, platelet aging and destruction, and may be released directly from megakaryocytes in platelet genesis (Nieuwland and Sturk, 2002).

3. Functions

Resting platelets circulate in the blood as single cells, but are rapidly able to change their structure, phenotype and release granule components in response to stimulation. Platelet activation occurs through ligand binding to a great variety of receptors on the platelet surface. This receptor binding signals exposure of fibrinogen binding sites and leads to platelet aggregation, shape change, receptor clustering, granule secretion, and synthesis of thromboxane. *In vivo* this process is thought to begin with the vWF receptor complex glycoprotein (GPIb-IX-V) and the collagen receptor GPVI. GPIb-IX-V recognises vWF after it has bound to collagen under shear. The weak binding allows platelets to roll on areas of damaged endothelium and initiates transmembrane signalling (Woulfe et al., 2002).

Exposure of fibrinogen binding sites and receptor clustering results in platelet–platelet aggregation and thrombus formation. Thromboxane synthesis and secretion of granule contents releases thromboinflammatory mediators at the site of vascular injury and alters expression of surface molecules, such as P-selectin, which facilitate further platelet adhesion and interaction with other cell types (see below). Therefore platelet activation, which may be triggered by a number of different signals, results in initiation of platelet–platelet aggregation through fibrinogen binding, release of thromboinflammatory mediators at the site of activation, and enables molecular interaction of the activated platelets with other blood cells, endothelial cells, and the coagulation system (Woulfe et al., 2002).

4. Interaction with other cells

Interaction of platelets with vWF and collagen leading to initiation of clot formation is well described (Ruggeri, 2002). More recently however, and while it remains incompletely understood, it has become increasingly evident that platelet attachment may also occur in conditions of vascular dysfunction the absence of endothelial denudation. Injured or inflamed endothelial cells downregulate antiplatelet prostaglandins and express molecules such as fibronectin, ICAM-1, endothelial P-selectin, E-selectin, integrin $\alpha_v\beta_3$, and vWF which promote platelet adhesion (Bombeli et al., 1998; Frenette et al., 1998).

Platelets that are activated and degranulated *ex vivo* immediately form heterotypic aggregates with leukocytes in a P-selectin dependent fashion upon reinfusion (Michelson et al., 1996, 2001; Huo et al., 2003). However, immediately after this both activated platelets and platelet-bound leukocytes disappear from circulation (Huo et al., 2003; Michelson et al., 1996), presumably localized to the endothelium (Huo et al., 2003), and it has been suggested that this platelet assisted localization of leukocytes to the endothelium may assist leukocyte involvement in plaque formation (Gawaz et al., 2005).

Monocytes and neutrophils form heterotypic aggregates with platelets initially via engagement of platelet surface P-selectin with leukocyte surface P-selectin glycoprotein ligand-1 (PSGL-1). The resultant intracellular signalling causes leukocyte surface expression of tissue factor and activation of Mac-1 (integrin αMβ2, CD11b/CD18). The activation-dependent conformational change in Mac-1 results in the binding of coagulation factor Xa (FXa) and fibrinogen with an increased adhesive phenotype (Barnard et al., 2005). During this interaction, platelets donate cholesterol to monocytes, and it has therefore been suggested that platelet adhesion may contribute to lipid macrophage differentiation (Mendelsohn and Loscalzo, 1988). Fig. 1 describes the impact of platelet interaction on leukocyte phenotype.