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The biology of the platelet with special reference to inflammation, wound healing and immunity

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1. ABSTRACT

While platelets have long been known to be essential for maintaining hemostasis in the vasculature, their role in tissue repair, inflammation and innate and adaptive immunity is a more recent science. The ability of platelets to attach to the vessel wall, form aggregates and promote fibrin formation, key elements of blood clotting, has been said to both favor and dampen inflammation, to fight infection and to assure an adequate immune response. To fulfill their different roles platelets often synchronize with leukocytes and cells of the immune system. But just as the molecular pathways of platelets in preventing blood loss can lead to arterial thrombosis and stroke if occurring in an uncontrolled manner, the failure to control inflammation can lead to sepsis and inadequate platelet function and can aggravate many major illnesses. This review is aimed to present a global picture of multifaceted platelet biology and platelet involvement in selected non-hemostatic events.

2. INTRODUCTION

Platelets are liberated in vast numbers from megakaryocytes (MKs), large multinucleate cells formed from hematopoietic stems cells (HSC) in a multistep process regulated by thrombopoietin (TPO) in the bone marrow (Figure 1) (1). Transcription factors control the many steps of MK maturation. After initial mononuclear cell proliferation, MKs undergo polyploidy: when mature, they migrate to the endothelial barrier of vascular sinuses and extend long processes termed proplatelets into the blood

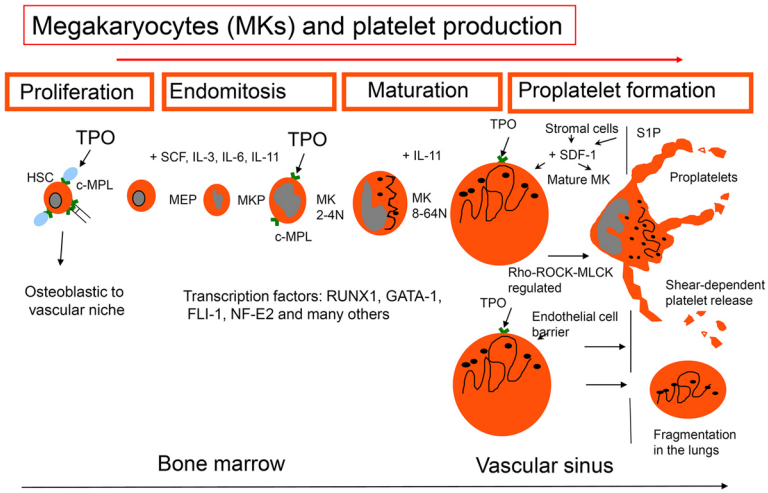

Figure 1. Cartoon featuring key steps in megakaryopoiesis and platelet production. Human stem cells (HSC) proliferate largely in the oteoblastic niche of the bone marrow under the influence of thrombopoietin (TPO) reacting with its receptor (c-Mpl). Under the influence of soluble factors including interleukins (IL) and stem cell factor (SCF), HSCs give rise to megakaryocyte-erythroid progenitors (MEP) and clonal megakaryocyte precursors (MKP) that multiply and form colonies. MKs have the specific characteristic of undergoing endomitosis multiplying their chromosome number many fold. The now large polyploidal cells then undergo an extraordinary maturation under the control of many transcription factors with the production of an extensive intracellular membrane system and multiple granule types. Having migrated to the endothelial cell barrier lining the vascular sinus, mature MKs interact with stromal cells and protrude large processes termed proplatelets into the vascular sinus under the regulation of many intracellular (e.g. Rho-ROCK and myosin light chain kinase (MLCK) phosphorylations controlling myosin-IIA activity) and extracellular signalling pathways (e.g. SDF-1, S1P). Proplatelets bud of platelets into the circulation or break into large fragments under shear. In an alternative pathway, the MKs themselves pass into the circulation and fragment under shear in the microvasculature of the lungs.

stream (2). Platelets then either bud off directly or large fragments disperse and divide in the circulation, a process accentuated in the lungs. Some MKs will also migrate directly into the vascular sinus and pass into the microcirculation of the lungs and fragment there (3). Other events in platelet biogenesis include division of dumb-bell shaped preplatelets and even multiplication of platelets themselves (4). Anucleate discoid platelets circulate in large numbers; the normal range is 150,000–400,000/μL of blood. Their primary role has long been established and is to assure hemostasis by preventing blood loss. For this, platelets possess a unique range of receptors, including for adhesion glycoprotein (GP) Iba (GPIbα) that as part of the GPIb-IX-V complex recognizes surface-bound von Willebrand factor (VWF), integrins α2β1, α5β1 and α6β1 that respectively interact with collagen, fibronectin (Fn) and laminin, while GPVI has unique properties in organizing the platelet response mainly to collagen and fibrin (Figure 2). Receptors for soluble agonists include $P2Y_1$ and $P2Y_{12}$ that bind adenosine diphosphate (ADP) while proteinase-activated receptor-1 (PAR-1) and PAR-4 coordinate the response to thrombin; other important receptors are those for thromboxane A_2 (TXA_2) and adrenaline all of which lock into a complex intracellular signaling network that controls the platelet functional response (5–7). On platelet activation, the high-density integrin aIIbβ3 changes from a bent to an extended conformation and binds fibrinogen (Fg) or other adhesive proteins to bring about platelet aggregation (8–9). The newly formed bridges hold platelets together and allow secondary interactions between other membrane GP pairs (eg. Ephrins and Eph kinases, semaphorins) that consolidate platelet-to-platelet cohesion and regulate thrombus stability and permeability within the aggregate (10–11). The αIIbβ3 integrin also binds fibrin; in fact, there is a greater binding strength with fibrin that will help the platelets to retract the fibrin clot (12).

Endothelial cells (EC) form a protective barrier and help maintain circulating platelets in a resting state by secreting prostacyclin (PGI_2) and nitric oxide (NO) that dampen their reactivity, but platelets come into play after EC loss or structural modification (such as during atherosclerosis or inflammation) (13). Due to their small size platelets tend to circulate close to the vessel wall at the edge of the blood stream helping them to support hemostasis; in fact, elevated flow itself can be activating (14). Attached platelets spread on the extracellular matrix, particularly collagen, secrete metabolites and release the contents of storage organelles (dense granules, a-granules); processes that promote thrombus formation and the ensuing tissue repair. Other membrane proteins such as platelet-endothelial cell adhesion molecule-1 (PECAM-1) negatively regulate platelet function and intervene in inflammation (15).

Transport of the anionic phospholipid, phosphatidylserine (PS) from the inner to the outer leaflet of the phospholipid bilayer makes the platelet membrane procoagulant. This Ca^{2+}-dependent process is optimal when thrombin and collagen act in

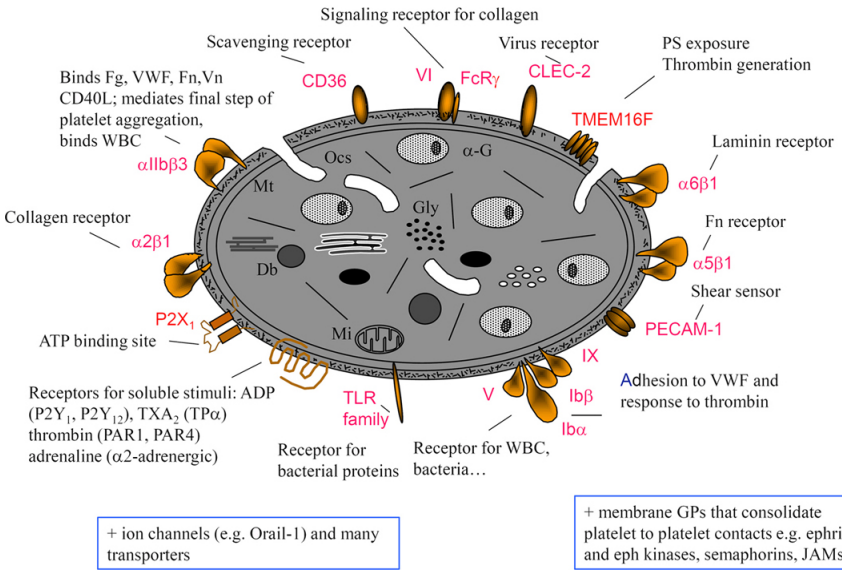

Figure 2. Cartoon showing the principal functional components of the platelet surface. Also identified are the major platelet intracellular organelles. Mt, microtubular band; α-G, α-granule; Db, dense body; Mi, mitochondrion; Ocs, open-surface canalicular system: Gly, glycogen

synergy; apoptosis and necrosis also mediators with loss of mitochondrial membrane potential a major factor in promoting PS expression (16). Platelets in the central core of the aggregate (or thrombus) are more tightly packed and undergo more extensive secretion than those in the outer shell; this consolidation regulates intra-thrombus solute transport and local thrombin activity (10–11). The latter catalyzes the transformation of Fg to the glue-like fibrin within the thrombus core where it promotes thrombus stability (17). It is also in the center of thrombi that activated platelets mostly secrete their newly formed metabolites and release storage pools of biologically active substances and proteins, with retraction playing a role in their extravasation. Activated platelets with formation of aggregates after atherosclerotic plaque rupture or uncontrolled embolization of platelet masses that severely perturb or occlude the circulation are at the origin of arterial thrombosis and stroke (18). Fibrin is essential for blood clotting and wound repair, its strands entrapping other blood cells with platelet aggregates acting as hubs within the fibrin network and ultimately mediating clot retraction (19). Paradoxically, adsorption of a layer of Fg onto fibrin on the surface of clots formed on collagen under flow confers anti-adhesive properties and limits thrombus growth (20). This Fg layer is extensible and incapable of transducing strong mechanical forces with the result that incoming platelets rapidly detach.

Platelets circulate for 7 to 10 days and are either used up in hemostasis or undergo programmed cell death through apoptosis with the balance between Bcl-xL and Bak constituting a molecular clock (21). In an alternate mechanism, platelets undergo glycosylation changes with aging and are removed from the circulation in the liver by way of the Ashwell-Morel receptor, a process that stimulates production of TPO in a feedback mechanism that masterminds platelet production (22). Inherited platelet abnormalities or acquired defects (including certain drugs, chemotherapy, viral or bacterial infections, autoimmune-mediated destruction) can result in a dramatic fall in platelet numbers (i.e. below 30,000/μL) and/or a loss of platelet function both of which will favor bleeding (23–25). In addition to their essential hemostatic role, evidence is accumulating that platelets also play fundamental roles in a wide spectrum of biological processes. They perform a housekeeping role by assuring vascular integrity, not only by physically blocking gaps in the endothelial cell layer but also by secreting proteins and substances that promote barrier function (26–27). Platelets intervene in tissue regeneration and angiogenesis, metastasis and tumor growth, inflammation, control of infection and innate immunity as well as controlling lymphatic vessel development (28–35). As well as receptors essential for the cell contact interactions bringing about thrombus formation, platelets also possess receptors unique for non-hemostatic events such as the toll-like receptors (TLR) that engage bacteria or C-type lectin-like receptor (CLEC)-2 that plays a major role in lymphatic vessel development (35–36). This selective review will now largely concentrate on describing the pleiotropic functions of platelets in non-hemostatic events.

3. PLATELETS AS A SOURCE OF BIOLOGICALLY ACTIVE PROTEINS AND METABOLITES

Certain features of a typical discoid anuclear platelet stand out (Figure 2). These include a delimiting

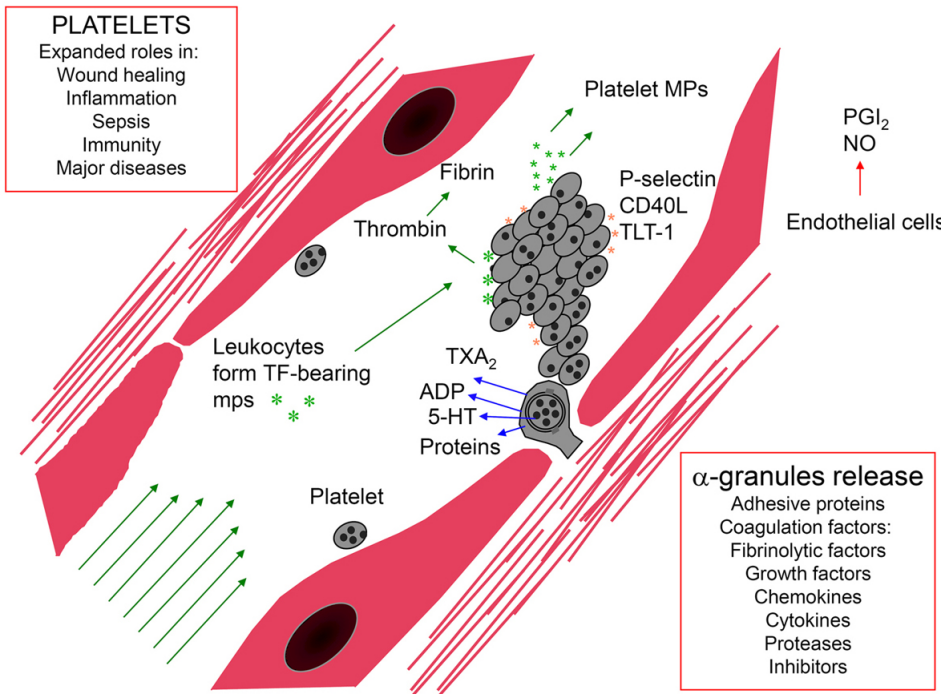

Figure 3. Schema showing thrombus formation under flow following a small vascular lesion and the autocrine release of metabolites and dense body contents from platelets. Highlighted are some of the principal non-hemostatic roles of platelets and the different categories of α-granule proteins given in detail in Table 1. Note the release of negative regulators (PGI_2, NO) of platelets from endothelial cells.

plasma membrane linked to an extensive intracellular open canalicular membrane system (OCS). Below the surface membrane is a microtubular network that interacts with an actin-rich cytoskeleton, while the cytoplasm contains mitochondria and a series of organelles that contain storage pools of bioactive molecules (19). Lipid metabolites such as TXA_2, whose formation is inhibited by aspirin (37), act in feedback mechanisms promoting the binding of adhesive proteins to the αIIbβ3 integrin and platelet aggregation. Sphingosine 1-phosphate (S1P) (see Figure 1 for MK maturation) stimulates mitogenesis and cell proliferation and is released from platelets during clotting; S1P is important in inflammation it favors Fn fibril assembly, endothelial barrier integrity and tissue factor (TF) expression in the vasculature (38). Lysophosphatidic acid and platelet activating factor (PAF) are other metabolites released. Another early response of the platelet, and a major subject of this review, is the release of the storage pools of biologically active agents from granules (Figure 3).

3.1. Dense granules

These small lysosome-related organelles (3 to 8 per platelet) contain serotonin (actively taken up by circulating platelets), ADP, ATP, GDP, GTP, polyphosphates, Ca^{2+} (itself a potential central regulator of wound healing) as well as small amounts of other amines such as histamine and dopamine. The dense granule membrane features transporters associated with the uptake and storage of their contents such as TPC2 (two-pore channel 2; Ca^{2+}), VMAT2 (vesicular monoamine transporter 2; serotonin) as well as membrane glycoproteins such as P-selectin, LAMP-2 (lysosomal associated membrane protein-2) and LAMP-3 shared with other organelles (19, 39). Dense granule release from platelets requires a complex secretory mechanism involving SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) proteins and many others involved in vesicular trafficking and late membrane fusion events required for exocytosis (40). ADP has a universal role in assuring stable platelet aggregation. In contrast, highly charged polyphosphates promote coagulation through initiation of the intrinsic pathway of coagulation and enhance fibrin clot structure; they provide an early link between platelets, coagulation and inflammation (41). Released serotonin stimulates vasoconstriction while increasing vascular permeability. Although the subject of debate, release from dense granules is thought to occur faster than from a-granules.

3.2. Alpha-granules

These storage organelles for proteins are abundant with 50–80 α-granules per platelet. They are formed from intermediate multivesicular bodies (MVB) originating from the trans-Golgi network in

Table 1. Platelet α-granule contents arbitrarily grouped in functional categories

Category	Protein	Function
Adhesive proteins	VWF + pro-peptide, *Fg*, Fn, Vn, TSP-1 and -2, laminin-8 (also α5-laminin subunit)	Cell contact interactions, hemostasis and clotting, extracellular matrix
Clotting factors and their inhibitors	Factor (F) V/Va, FVI, FVII, FVIII*, FX, FXI, FXIIIa and b subunits, TF*, prothrombin, multimerin 1, protein S, high-molecular weight kininogen, protease nexin-1 and-2 (amyloid β A4 protein precursor), TFPI, protein C inhibitor	Thrombin production and clotting. Wound healing
Fibrinolytic factors and their inhibitors	Plasminogen/plasmin, PAI-I, urokinase plasminogen activator, α2-antiplasmin, α2-macroglobulin, histidine-rich glycoprotein, thrombin-activatable fibrinolysis inhibitor	Plasmin production, fibrinolysis and vascular modeling
Proteases and anti-proteases	MMP-1–4, -9, -14, ADAMTS-13, ADAM-10 (a-secretase), ADAM-17, TIMPs 1–4, C1 inhibitor, α1-antitrypsin, α2-antitrypsin, α2-macroglobulin, granzyme B*, bradykinin	Angiogenesis, vascular modeling, regulation of coagulation, regulation of cellular behaviour
Growth and mitogenic factors	PDGF (A, B and C), EGF-2, HGF, SCUBE1 (EGF-like), IGF-1, IGF binding protein 3, VEGF (A-D), FGF-2, bone morphogenetic protein-2, -4, -6, -11, GDF-15, gremlin-1, gas6**, CTGF	Chemotaxis, cell proliferation and differentiation, angiogenesis
Cytokines, chemokines and others	TGF-β1, -β2, -β4, IL-1α, IL-1β ***, IL-2–4, -6–8, -10, -11, TNF-α, TNF-β, IFNg, CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL7 (MCP-3), CCL14, CCL15 (MIP-5), CCL17, CCL19 (MIP-3b), CCL20 (MIP-3α), CCL21, CCL22 CXCL1 (GROα), CXCL2 (MIP-2α), CXCL3 (MIP-2β), CXCL4 (PF4), CXCL4L1, CXCL5 (ENA-78), CXCL6, NAP-2, CXCL7 (PBP or β-thromboglobulin that gives rise to NAP- 2 and connective-tissue-activating peptide III), CXCL8 IL-8), CXCL12 (SDF-1α), granulocyte-macrophage colony-stimulating factor (GM-CSF, CSF-1), CXCL16, TNFSF14 TPO*, angiopoietin-1 and 2, angiopoietin-like protein 2, HMGB1, IL-6sR, osteonectin, bone sialoprotein, Dkk1, Wnt3a, osteoprotegerin, BDNF, g-interferon protein 10 (IP-10), *endostatin (proteolytic fragment of collagen), angiostatin (proteolytic fragment of plasminogen),* oncostatin M, angiogenin	Regulation of angiogenesis, cellular proliferation and differentiation, chemotaxis, vascular modeling, cellular interactions, bone formation
Anti-microbial proteins	Many chemokines and truncated derivatives e.g; thrombocidins (from CTAP-III and NAP-2) and kinocidins****, Human beta-defensin-1, -2, -3*****, thymosin-β4	Bactericidal and fungicidal properties
Miscellaneous proteins	Serglycin (secretory granule proteoglycan core), chondroitin 4-sulfate, syndecan-4, *albumin, IgG, A and M,* α-actinin-1, -2, -4, thymosin-β4, amyloid beta (A4) precursor, disabled-2, complement C3 and C4 precursor, complement factor D, factor H, C-reactive protein, bile salt-dependent lipase, substance P, reelin, clusterin, autotaxin, PDI)******, ERp5, ERp57, cyclophilin A, *cellular prion protein*	Various functions
Membrane glycoproteins	αIIbβ3, αvβ3, GPIb, PECAM-1, ICAM-2, semaphorin 3A, semaphorin 4D, PLEXIN-B1, extracellular matrix metalloprotease inducer (EMMPRIN), receptors for primary agonists, P-selectin, TLT-1, JAM-1, JAM-3, claudin-5, PSGL, CD40, CD40L, Apo3-L, TRAIL, gC1qR, FasL, Tie-2, mer, major histocompatibility complex proteins, beta-2-microglobulin, Siglec-7, hyaluronidase-2, CD39, CX3CL1, CXCR4, galactin-1, -8, TLR-1, -2, -4, -6.	Platelet aggregation and adhesion, endocytosis, of proteins, inflammation, thrombin generation, platelet-leukocyte and platelet-vascular cell interactions, immune modulation, apoptosis

This list of proteins is as complete as possible but does not include many additional proteins identified by proteomic analyses (reviewed in Burkhardt et al, 2014). Proteins captured by endocytosis are in italics, others are presumed synthesized in MKs. The abbreviations for many proteins are written in full in the text, space does not allow the addition of references for each protein. *Proteins whose presence is controversial; **Gas6 is present in mouse but not human platelets. ***IL-1β is primarily released in microparticles after spliceosome-dependent synthesis. ****C-terminal peptides of CXC chemokines, *****human beta-defensins are abundant in platelets and while their presence in a-granules cannot be excluded their release can also occur from the cytoplasm through staphylococcus aureus-induced pores . ******PDI is thought to occur in a separate intracellular organelle called T-granules whose relationship to a-granules is uncertain; the same may apply to the TIMPs.

maturing MKs (40, 42). Some MVB and α-granules may contain smaller vesicular structures called exosomes that are enriched in LAMP-2 and secreted intact; their significance is largely unknown (40). Table 1 details just how comprehensive is the platelet storage pool of proteins. Here, chosen proteins are arbitrarily grouped into functional categories, notwithstanding many proteins have several, even seemingly opposing functions. Proteomics have revealed just how wide and diverse is the α-granule content with several hundred proteins identified (reviewed in 43). This has been narrowed down to 124 proteins that were said to have significant release after exclusion of proteins released by spontaneous platelet lysis (44). Mostly, stored proteins are synthesized in MKs and traffic in endosomes to MVB and developing granules; nevertheless, some proteins are captured by MKs or platelets from their environment by endocytosis (e.g. Fg, albumin, IgG) (Table 1) (42). For example, Fg is captured by the αIIbβ3 integrin and recent studies have shown a role for adenosine 5'-diphosphate-ribosylation factor 6 (ARF6) as a regulator of integrin trafficking, a process that requires clathrin and which also plays a role in clot retraction and platelet spreading (45). The α-granule proteins are grouped in Table 1 within

categories such as adhesive proteins, clotting and fibrinolytic factors, growth factors, cytokines and chemokines composed of small CXC-ligands (CXCL) or CC-chemokine ligands (CCL), proteases and their inhibitors, and anti-microbial proteins. Ca^{2+} and Mg^{2+} are also present in a-granules that contain acidic glycosaminoglycans (mainly chondroitin-4-sulphate) localized to distinct domains (or cores) where they concentrate basic proteins such as platelet factor 4 (PF4, CXCL4). The granule membrane contains not only intrinsic GPs of the plasma membrane (e.g. αIIbβ3), but also more selective components such as P-selectin, trem-like transcript-1 (TLT-1), semaphore 4D and CD40L whose surface expression confers new properties to the activated platelet allowing platelet-leukocyte tethering or platelet interactions with other cells.

Adhesive proteins abundant in the α-granule storage pool include VWF, Fg, Fn, vitronectin (Vn) and thrombospondin-1 (TSP-1) (Table 1) all of which participate in platelet surface contact interactions even if Fg plays the major role in aggregation (8–9). Fibrillar cellular Fn in the vessel wall is an excellent substrate for thrombus formation, supporting platelet interactions through the α5β1integrin, GPIb, GPVI and, quite unexpectedly, toll-like receptor 4 (TLR4) (46–47). Special mention should be made of TSP-1, one of the most abundant α-granule proteins; TSP-1 plays a major role in thrombus stability and clot retraction (48). Adhesive proteins may also act directly as mitogens or they may promote mitogen activity of growth factors. The α-granules are a rich source of coagulation factors (F) V and XI that on secretion promote thrombin formation on the platelet surface. However, α-granules also contain a number of inhibitors of coagulation (e.g. tissue factor pathway inhibitor (TFPI), protease nexin-2) and of fibrinolysis (plasminogen activator inhibitor type I, PAI-1) (Table 1). This illustrates the fundamental enigma of platelet α-granules that store proteins with opposing effects. Platelet α-granules also have surprises, such as the presence of brain-derived neurotrophic factor (BDNF), synthesized by MKs and released on secretion (49). Platelets are a major source of BDNF suggesting a role for them in the brain. Although lacking a nucleus, platelets contain a spliceosome (mRNA splicing machinery) that permit them to translate mRNAs into protein after platelet activation; two prominent examples are interleukin-1β (IL1β) that has a prominent role in inflammation and TF (50–51). However, these proteins are mostly synthesized after secretion has occurred and may have an alternative secretory pathway perhaps being liberated in microvesicles (see Section 4).

Proteins were for long assumed to target to α-granules randomly during granule biosynthesis. However, Italiano *et al* (52) localized pro-angiogenic proteins such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) and angiopoietin-1 in subpopulations of α-granules distinct from those containing anti-angiogenic proteins such as endostatin, angiostatin, TSP-1, PF4 and PAI-1 both in platelets and in MKs; they also expanded earlier works by showing that granule cargos were released with different kinetics. However such concepts have been challenged. By quantitative immunofluorescence mapping, Kamykowski *et al* (53) found little to indicate co-clustering of proteins within the α-granules. Although confirming kinetic heterogeneity, a time-dependent analysis of protein release from platelets stimulated by different agonists showed much overlap in secretion of proteins of opposing functions (54). It was suggested that platelet secretion is a stochastic process potentially controlled by factors such as cargo solubility, granule shape, and/or granule-plasma membrane fusion routes. High-resolution and scanning transmission electron microscopy (STEM) showed granule cargos to be compartmentalized zonally but within the same organelle while three-dimensional images obtained by cryo-electron tomography showed α-granules with microvesicular and tubular internal structures consistent with a three-dimensional spatial organization (55–56).

Heterogeneity was confirmed however when tissue inhibitors of metalloproteases (TIMPs) were localized to granules distinct from those containing VWF; often these were present close to the plasma membrane and even within filopodia (57). Significantly, platelets from patients with an inherited disorder of α-granule production (gray platelet syndrome) failed to label for VWF while normally containing TIMPs. Another secreted cargo stored in a specific compartment discrete from α-granules and labeled T granules by the authors, is protein disulfide isomerase (PDI) that on secretion associates with αIIbβ3 and helps stabilize a fibrin clot (58–59). PDI is the archetypal member of a large family of thioredoxin-like proteins of the endoplasmic reticulum (ER) of which platelets contain several members that also include ERp57 and Erp5 (60). These family members known for their role in oxidative folding and disulfide exchange intervene in thrombus formation and fibrin generation in which they appear to have non-redundant roles. They are also active players in neutrophil recruitment to the vessel wall in inflammation. Recent studies show that PDI and ERp57 are localized close to the inner surface of the plasma membrane within the dense tubular system (derived from MK smooth ER) and on platelet activation are mobilized by a process that requires actin polymerization and membrane/cytoskeletal reorganization (61); an organization similar to that seen for some TIMPs (57).

Platelet release of α-granule constituents requires docking and fusion of the granule membrane with

either the plasma membrane or an internal channel that opens to the surface. As for dense granules, exocytosis heavily involves vesicle- and plasma membrane-bound SNARE proteins and their chaperones in which vesicle-associated membrane protein (VAMP) -3, -7 and -8, synaptosome associated protein-23 (SNAP-23), Munc13–4, syntaxin-11 (STX-11), STX-BP5, Munc18–2 and Rab27b are prominent players (40, 62). STEM tomography has revealed another mechanism in that many α-granules liberate their contents through tubular extensions reacting directly with the plasma membrane; this can also involve the OCS membranes that join independently with the plasma membrane (56). Differential sorting of α-granules has also been shown, with granules labeling for VAMP-7 moving to a more peripheral localization during platelet spreading allowing it to play a role in cytoskeletal remodeling while a novel mechanism involving VAMP-8-dependent compound granule fusion has also been proposed (63–65).

A brief mention should also be made of metalloproteases (MMPs) of which platelets are a rich source containing MMP-1–4, -9, -14, ADAM (a disintegrin and metalloprotease)-10 and -17 and ADAMTS-13 (ADAM with thrombospondin type I repeats), among others (Table 1). ADAMTS-13 is a negative regulator of thrombosis and inflammation acting by cleavage of hyperactive large VWF multimers (66). While MMPs are often said to localize in α-granules, their presence in other non-identified organelles, as seen for the TIMPs, cannot be ruled out (57). The ubiquitous MMPs have many roles that include tissue remodeling (67). ADAM-10 and ADAM-17 are largely responsible for the cleavage of platelet surface receptors on platelet activation leading to the loss of a large part of the extracellular domains of GPIbα, GPVI and P-selectin as part of what is often called “sheddase” activity and therefore regulating platelet function (68). Localization of the sheddase enzyme is key and is under tight control, an example is the cleavage of GPVI by ADAM-10 where members of the Tspan C8 subgroup have been shown to mediate ADAM-10 intracellular trafficking and enzyme activity. In platelets, the interaction of Tspan14 with ADAM10 via its large extracellular loop provides specificity for the collagen receptor, GPVI (69).

In summary, differentially packaged and segregated proteins have different ways of reaching the platelet surface while the spatial localization of the granules, determined by VAMP isoforms, and the size of both the individual secreted proteins and the fusion pores will determine diffusion rates in response to different stimuli. Finally, the three-dimensional structure of thrombi in the vasculature and the extent of integrin-based clot retraction will control the extravasation of secreted and plasma borne molecules in wound healing and inflammation (35). Similar mechanisms occur in arterioles and venules although surprisingly it is in the latter that platelets exercise a greater role compared to fibrin and thrombin with secreted ADP playing a key role (70). Factors that control fibrin organization in thrombi and their microelasticity under flow include (i) the nature of the exposed surface generating thrombus formation, (ii) thrombin generation and TF expression both inside and outside the thrombus, and (iii) shear rate, lower shear forces favoring fibrin coverage of the thrombus.

3.3. Lysosomes

These are granule stores for enzymes such as cathepsins D and E, elastase, β-glucuronidase and acid phosphatase. Their membranes resemble dense granules in expressing LAMP-2 and LAMP-3. Platelets contain a constitutively active autophagy pathway that is essential in maintaining cellular homeostasis and which is activated further on platelet response to stimuli (71). Autophagy involves the constitution of autophagosomes containing protein complexes (perhaps the best known of which is light chain 3II, a microtubule derivative) able to fuse with lysosomes.

4. THROMBIN GENERATION AND MICROPARTICLE RELEASE

Thrombin generation is key for blood clotting and is mediated by the extrinsic and intrinsic pathways of coagulation. Activated platelets provide a catalytic surface that accelerates thrombin production. Platelet stimulation by strong agonists leads to increases in cytosolic Ca^{2+} and the surface exposure of phosphatidylserine (PS) (72). Interestingly, procoagulant platelets form distinct patches in a growing thrombus (73). PS expression on platelets allows the binding of coagulation factors from plasma or after autocrine secretion with the rapid formation of an activated factor Xa/Va complex that transforms prothrombin into thrombin (74). Thrombin itself is a powerful mitogen. However, its main immediate role is to generate fibrin. Apoptotic or necrotic pathways also lead to Ca^{2+}-dependent PS exposure and procoagulant platelets, for example through cyclophilin D-dependent mitochondrial permeability transition pore formation and loss of energy potential, processes that can occur within the developing thrombus (16, 75). Ballooning of procoagulant platelets with cytoskeletal disruption has been reported with membrane GPs and adhesive proteins retained within large patches (76–77). Intriguingly, such findings only apply to subsets of the total platelet population; nevertheless, an increased presence of procoagulant cells can tip the balance from hemostasis to thrombosis. PS-expressing platelets that retain procoagulant and serotonin-derived adhesive proteins are often referred to as “coated” platelets; their levels are often increased after

trauma, for example after brain injury and they have been implicated in stroke (78).

PS expression is also associated with the release of small membrane-bound microvesicles, commonly known as microparticles (MPs), from platelets (72, 79). Procoagulant in nature, they bud off in large numbers from the platelet surface following calcium-dependent uncoupling of the plasma membrane from the underlying cytoskeleton. MPs play essential functions in cell-cell communication and transport of mediators and are at the nexus between inflammation, immunity and thrombosis (79–80). Procoagulant in nature, MPs are elevated in a variety of immune disorders including systemic lupus erythematosus (SLE), Sjorgen's syndrome, multiple sclerosis, antiphospholipid syndrome and autoimmune disease of which rheumatoid arthritis is a prominent example (81–82). MPs are functionally active, they express P-selectin enabling them to react with leukocytes and this is of biological significance. In one example, the expression of 12-lipoxygenase leads to the release of 12(S)-hydroxyeicosatetranoic acid that in turn promotes MP internalization by neutrophils which with soluble phospholipase A2-IIA release promotes inflammation (83). Quite surprisingly, platelets can also release mitochondria, both within MPs and as isolated organelles (84). Degradation of the mitochondrial membrane by soluble phospholipase A2 also leads to the release of inflammatory mediators; mitochondria themselves can also bind to neutrophils.

5. PLATELETS AND BLOOD VESSELS

Platelets through the formation of the hemostatic plug prevent blood loss and help restore the structure of blood vessels after injury (see Section 1. Introduction). This involves tissue repair while platelets play a major role in angiogenesis both in damaged tissues and in tumors (85, 86). They also play a role in vascular disease with atherosclerosis the prominent example.

5.1. The repair process

Essential for maintaining vascular integrity, collagens, proteoglycans and adhesive proteins such as Fn (often organized in a fibrillar form) are major constituents of the subendothelial matrix; they provide a molecular scaffold for adhering platelets as well as fibroblasts and incoming cells at injured or inflamed sites (47, 87). Thrombus growth brings in further platelets that secrete their panoply of growth factors, cytokines, chemokines and active metabolites (Table 1). Proteins such as VEGF, PDGF a/b and c isoforms, FGF, hepatocyte growth factor (HGF), epidermal growth factor (EGF), connective tissue growth factor (CTGF) and IGF form chemotactic gradients around the lesion by directly binding to matrix components or to newly generated fibrin (19, 42). Some interact directly with vascular cells; for example, VEGF binds to its endothelial receptor (VEGF-R2) and induces signaling that leads to vessel relaxation and vasodilation (reviewed in 47). More nutrients arrive with the increased blood flow and recruitment of white blood cells is facilitated by platelet/leukocyte crosstalk and macrophage development under the influence of macrophage inflammatory proteins (MIPs) (see also Section 6. Inflammation). In another example, secreted and activated transforming growth factor-β1 (TGF-β1) recruits inflammatory cells into the wound area and stimulates fibroblasts to produce connective tissue, a process that if not controlled can result in fibrosis (89). Fibrinogen itself can enhance wound closure by favoring cell proliferation and migration and it forms mixed fibrils with Fn, a substrate for αvβ3 on fibroblasts (90). Fibrin is another key player in wound healing (91). Fibrinolysis produces fibrin degradation products that also attract leukocytes and aid the transition between inflammation and tissue repair. The benefits of the now widespread therapeutic use of platelet-rich plasma clots are obtained through an enhancement of this natural process (19).

The liver provides a special example of how platelets intervene in organ repair as it has a unique regenerative capacity. Platelet released serotonin and growth factors (e.g. HGF, VEGF and IGF) enhance this process (92). However, alternative mechanisms for platelet involvement are yet to be ruled out and these include roles for (i) ADP and other released molecules (ii) a direct transfer of mRNA to liver cells and (iii) neutrophil attraction secondarily favoring an inflammatory response (reviewed in 93).

5.2. Angiogenesis

Restoring vascularization at sites of vessel injury relies heavily on platelets and their released proteins to promote recruitment, growth and proliferation of endothelial and other vascular cells (94). Released factors such as VEGF, bFGF and PDGF, enhance not only endothelial cell proliferation but also later events such as endothelial tube formation and sprouting of new vessels (95). Another key protein is stromal cell derived factor-1 (SDF-1), an α-granule stored chemokine that by binding to its receptors CXCR4 and CXCR7 on progenitor cells or mesenchymal stem cells enhances their recruitment to the site of vascular lesions (96). The whole process is regulated in coordination with growth factors and inhibitors released from endothelial and vascular cells with a role for endothelial cell membrane signaling molecules such as PEAR1 (97). Enigmatically, the platelet content of angiogenesis regulators was selectively increased in mice bearing tumors suggesting that they are also taken up from plasma (98). There is also the apparent contradiction, yet to be resolved, that

platelets also possess and secrete anti-angiogenic factors such as endostatin, PF4, TSP1 and the TIMPs that may counterbalance the effect of the pro-angiogenic mediators (99). PF4 (CXCL4) is perhaps the best studied of these as it also has immune-modulatory properties. PF4 binds with high affinity to heparin and to heparin-like molecules on the endothelial cell surface and negatively regulates angiogenesis by inhibiting VEGF and FGF as well as blocking the cell cycle (100).

Platelet membrane constituents also intervene in favoring angiogenesis. Optimal tube formation from endothelial colony-forming cells, and particularly the number of branching points, requires the presence of tetraspanin (Tspan), CD151 (CD40L) and the laminin-binding integrin α6β1on platelets and endothelial cells (101–102). Tspans are functional partners of integrins and segregate them into plaques or domains to increase their density; they are widely present in platelets, endothelial and tumor cells (103–104). Tissue factor is the initiator of the extrinsic pathway of coagulation; it also plays a key role in angiogenesis and wound healing (105). Whether circulating platelets actually possess TF is unclear; however, they can take it up from monocytes and circulating MPs in a P-selectin dependent mechanism while activated platelets can make it through pre-mRNA splicing by using their spliceosome (51). A role for PDI and perhaps PS in modulating the initiation of TF activity has been proposed (60).

5.3. Atherosclerosis

Atherosclerotic plaque formation provides a link to the following Section as it can be considered as chronic inflammation and the reader is invited to consult specialist reviews for more detailed information on this much-studied condition (31, 47, 88, 106–108). Suffice to say here that activated platelets secrete biologically active molecules such as PF4 and RANTES (regulated on activation, normal T cell expressed and secreted) that alter the differentiation of T cells and macrophages, increase uptake of oxidized LDL and stimulate monocyte recruitment. SDF-1α is important for regenerative processes while platelet surface glycoproteins including αIIbβ3, GPIbα, P-selectin, junctional adhesion molecule-A/C (JAM-A/C) and CD40/CD40L are crucially important in regulating platelet interaction with endothelial cells, leukocytes, dendritic cells and matrix components involved in plaque formation (109–110). Platelets affect cholesterol metabolism by interacting with and capturing LDL particles and by contributing to the formation of lipid-laden macrophages and foam cells (111–112).

6. PLATELETS AND INFLAMMATION

As seen in atherosclerosis, platelets participate actively in inflammation by promoting leukocyte accumulation at inflamed sites and aiding the attached cells to migrate within the vessel wall (113). At the same time they help prevent hemorrhage at sites of neutrophil diapedesis and disruption of endothelial cell junctions (114–115). GPIb plays an important role in platelet recruitment following release of ultralarge VWF strings from the Weibel-Palade bodies of activated endothelial cells at inflamed sites and GPVI mediates activation of platelets by exposed collagen (reviewed in 31). P-selectin expression helps arrest platelets under conditions of high flow and favors white blood cell recruitment to inflamed sites. The role of platelets extends well beyond the vascular system. For example, notwithstanding the blood-brain barrier, platelets influence central nervous system repair and can regulate neuro-inflammation and influence regenerative processes – essentially by interacting with stem/progenitor cells (116).

6.1. Cell survival and apoptosis

It is pertinent to question how platelets are capable of modulating the balance between cell survival and apoptosis in tissues. SDF-1 acting with serotonin, ADP and S1P favors cell survival. A number of tumor necrosis factor-α (TNF-α-related apoptosis regulators are secreted from platelets including CD40L, soluble Fas Ligand (sFasL) which with membrane-bound FasL promote apoptosis as a defense mechanism against inflammation (117). Another platelet-expressed protein, TNF-related apoptosis-inducing ligand (TRAIL) regulates apoptosis in cells including fibroblasts, smooth muscle cells, neutrophils and monocytes (118). ATP release from dying cells and damaged tissues acts as a danger signal being part of a damage-associated molecular pattern (DAMP) that start and perpetuate a noninfectious inflammatory response; ATP release from platelets in a growing thrombus can potentiate this process (5). Released ADP also favors platelet/leukocyte interactions. Polyphosphates may interact with endothelial cell P2 receptors and amplify their inflammatory response (reviewed in 5). They also increase vascular permeability and promote edema by favoring bradykinin production (119).

6.2. Interplay between platelets, leukocytes and vessels

As we have already mentioned, inflammation involves close interplay between platelets, leukocytes (including cells of the immune system) and the vessel wall (see Section 1. Introduction). GPIb-mediated platelet adhesion helps trap leukocytes at sites of inflammation; P-selectin on the now activated platelets binds to its counter receptors, including P-selectin glycoprotein ligand (PSGL)-1, on white blood cells (28–31, 113). Studies in mice show how platelet-released serotonin also promotes the recruitment of neutrophils to sites of

acute inflammation (120). Under flow, neutrophils roll along inflamed vessels, stop and then migrate into perivascular tissues. Interaction of neutrophil ligands with endothelial P- and E-selectins exposed at inflammatory sites can also lead to rolling while intercellular adhesion molecule (ICAM)-1 on endothelial cells can also bring about neutrophil arrest. Signaling through chemokine receptors leads to activation of β2 integrins on leukocytes; with CalDAG-GEFI, Rap1, kindlin-2 and -3 and talin all having essential late-signaling roles in integrin activation and transformation to an extended high affinity conformation, a step essential for stable cell adhesion, cytoskeletal reorganization and cell migration (121–122). In a major development it was even proposed that recruited neutrophils scan for platelets and that the involvement of platelets is a two-way process (123). Neutrophil polarization within venules led to a protruding domain that continued to engage activated platelets in the blood stream. Intriguingly, the active form of the Rho GTPase, Cdc42, was shown to negatively regulate chemokine-induced integrin activation (124). Cdc42 is activated by growth differentiation factor 15 (GDF-15), related to the TGF-β family. In fact, recent studies have shown that both GDF-15 and TGF-β1 inhibit chemokine-triggered integrin activation through heterodimers of TGF-β receptor 1 (TGF-βR1) that in turn lead to Cdc42 activation (125). The latent form of TGF-β1, a major protein released from platelet α-granules, can be activated by shear (126). This raises the intriguing possibility that platelet cytokines and shear help regulate integrin activation and thrombus growth at inflamed sites and provides a further example of the complexity of the roles of α-granule proteins.

The diversity of biologically active substances secreted from platelets cannot be over stated. Platelets rapidly synthesize pro-inflammatory metabolites including TXA_2 and PAF while ADP, ATP, polyphosphates, serotonin and histamine are released from dense granules (5, 127). As well as the growth factors already outlined in this review, platelets release a range of cytokines and chemokines to their environment (Figure 3) (Table 1). As well as PF4, platelet basic protein (CXCL7) and RANTES favor immune and inflammatory processes. As an example, neutrophil-activating peptide-2 (NAP-2, a proteolytic derivative of CXCL7) attracts immune cells to traverse the thrombus and enter the vessel wall (34, 128). Other proteins of special interest in terms of inflammation are IL-8, macrophage migration inhibitory factor (MIF), growth-regulated oncogene-α (Gro-α), epithelial activating protein-78 (ENA-78) and monocyte chemoattractant protein-3 (MCP-3). Significantly, many of these proteins are involved in atherosclerotic plaque formation (discussed in 107). Platelet immunoreceptor tyrosine-based activation motif (ITAM) signaling is critical for securing vascular integrity for inflammatory bleeding occurred in the skin and lungs of platelet-depleted mice or those transfused with antibodies blocking platelet GPVI, CLEC-2 or SLP76 (129). In fact platelets secure inflamed vessels and play a positive vascular protective role during the inflammatory response.

7. PLATELETS AND INFECTIONS, NET FORMATION AND SEPSIS

Increased bleeding is seen in inflammatory states such as immune-complex-induced vasculitis and LPS-induced lung inflammation when the platelet count is low (34, 114). Platelets are also active in promoting rheumatoid arthritis (RA) through release not only of biologically active proteins from a-granules but also by way of MPs (81, 130). MPs act as carriers of immune complexes (ICs) consisting of immunoglobulin and complement (C) with the MPs themselves contributing the autoantigen often identified as a citrullinated protein (e.g. vimentin and Fg) (82). A mixture of MPs and ICs from RA fluid was shown to be inflammatory, stimulating neutrophils to secrete leukotrienes. It was suggested that MPs enter the synovial fluid through gaps between endothelial cells in the inflamed vasculature and then undergo citrullination and form the immune complexes. In sterile inflammation, cell death can lead to release of DAMPs (131). These stimulate the immune response to remove dead cells but can have inflammatory off-target effects.

7.1. Anti-microbial and anti-viral roles in host defence

A special and increasingly recognized function of platelets is in host defense both in circulating blood and at vascular sites of lesions such as in endocarditis (127, 132). Platelets can be involved in counteracting microorganisms such as in malaria, where they bind to infected red cells and release proteins that kill intra-erythrocytic parasites (133). Platelets contain a variety of receptors recognizing pathogens including members of the toll-like receptor family (TLR1–7; -9) whose engagement with bacterial targets leads to platelet activation and release of microbicidal proteins and cytokines (Table 1) that promote recruitment of circulating inflammatory cells whose role is bacterial destruction (134). Other platelet receptors involved in bacterial clearance are GPIb-IX and αIIbβ3 that bind Staphylococcal proteins among others and and FcγRIIA (see 33). Platelets bind to Escherichia coli (E coli) through FcγRIIA binding to IgG coating the bacteria; this leads to platelet aggregation by a secretion- and αIIbβ3-dependent mechanism (135). Inflammation drives thrombosis in the liver after Salmonella infection and does so in a TLR4-dependent cascade via ligation of C-type lectin-like receptor-2 (CLEC-2) on platelets by the membrane

glycoprotein, podoplanin, on monocytes and kupffer cells (136). In contrast, platelets also participate in bacterial clearance by collaborating with Kupffer cells through the recognition of VWF on the Kupffer cells by GPIb (137). Some cytokines released from activated platelets have direct microbicidal activities (Table 1) including PF4, platelet basic protein (PBP, CXCL7), thymosin-β4 and RANTES. Thrombocidins (TC-1 and -2) are small C-terminal proteolytic derivatives of CXCL7 such as NAP-2 (138).

Platelets can also directly bind and phagocytose many types of virus while thrombocytopenia accompanies many viral infections (132). A specific example is the binding of human immunodeficiency virus (HIV)-1 virus to CLEC-2 (also called DC-SIGN) followed by its internalization while interestingly some viral surface proteins exhibit molecular mimicry with platelet αIIbβ3 (139–140). Strikingly the Dengue virus enters the platelet allowing replication of the positive sense single strand RNA genome of the virus thereby aggravating infection (141). Even influenza virus reacts with platelets with the membrane-bound hemagglutinin of the virus mediating antibody and complement-dependent lysis of platelets (142).

The complement system plays a key role in inflammation and immunity. Platelets store and secrete elements of the complement (C) cascade (C3, C4 precursor) from their α-granules as well as proteins that regulate complement activity (C1 inhibitor, factor H) (143). In this context, properdin-mediated C5a production enhances stable binding of platelets to granulocytes, a process tightly regulated by factor H (144). Complement proteins have a reciprocal relationship with platelets that activate the complement system while complement proteins can also activate platelets (145). Interestingly, polyphosphates released from platelet dense granules mimic heparin by acting as a template for C1 inhibitor and thus are an important regulator of complement activation (146).

7.2. NET formation

NETs are webs of histone-modified nuclear material extruded from activated neutrophils. Platelet engagement through platelet-neutrophil interplay has a key role in NET formation, an event essential for optimal host response to major infection but which can also have harmful effects such as promoting microvascular and deep vein thrombosis (DVT) - both in sepsis and as a more confined example, transfusion-related acute lung injury (147–149). A TF-dependent coagulant state promotes fibrin formation in large vessels in DVT (see 150). Despite much evidence that neutrophils play important roles in venous thrombosis, the role of platelets and FXII has been recently emphasized (151). Neutrophils kill and remove bacteria and viruses at sites of infection and the NETs consisting of scaffolds of histone-containing chromatin fibers, serine proteases and released reactive oxygen species allow this to be done on a large scale. But an excess of histone release can be harmful and may contribute to lethality in sepsis. NET formation has also been observed in pre-eclampsia, vasculitis and systemic lupus erythematosus and its associated nephritis while antibodies against NET components (including DNA) promote the pathology of autoimmune disease (see 152). The negatively charged DNA of NETs initiates the contact pathway of coagulation and thrombin generation (153). Histones also promote thrombin generation in a platelet-dependent manner (154).

7.3. Sepsis

An extreme condition linked to uncontrolled inflammation and organ dysfunction; sepsis is associated with a high incidence of thrombosis and mortality (155–156). Platelets have a major influence on the host response during sepsis. I have dealt with the role of platelets in bacterial clearance, but it should not be forgotten that many of their receptors are shared with endothelial and other blood cells, one example being αvβ3. Staphyloccus aureas, one of the primary etiologic agents of sepsis also causes endothelial cell dysfunction and death through initial Fg-dependent binding to αvβ3 (157). Endothelial cell activation or damage can lead to VWF deposition thereby promoting platelet accumulation (158). Platelet accumulation in inflamed tissues accelerates immune cell recruitment and the onset of organ dysfunction. In a mouse model of sepsis induced by cecal ligation and double puncture, neutrophil infiltration in the lungs was reduced after platelet depletion suggesting that platelets play a role in neutrophil activation during inflammation (159). The association of sepsis with disseminated intravascular coagulation (DIC) can lead to a fall in platelet count and impaired vascular integrity and favorise edema, shock and organ failure with inflammatory and thrombotic responses favored by thrombin generation and a late stage involvement of complement activation products (160). Platelet activation through FcγRIIA-dependent and independent mechanisms with αIIbβ3 activation may also favor thrombosis, a major cause of death in sepsis and meningitis. Venous thrombosis is aided by decreases in the levels of thrombomodulin on endothelial cells and a reduction in circulating protein C (161).

Thrombocytopenia is associated with greater mortality and platelet transfusion has been shown to be protective by inhibiting macrophage-dependent inflammation (162). In this context, the Ashwell-Morel receptor for desialated proteins in the liver has been proposed to mitigate the lethal coagulopathy of sepsis (163). As an example, infection with Salmonella pneumoniae can trigger DIC with consumption of

coagulation proteins and platelets leading to fibrin deposition in multiple organs. The Ashwell-Morel receptor provides an adaptive response to bacterial infection by removing desialated proteins and cells from the circulation. Sepsis is a progressive systemic inflammatory condition and the kallikrein/kinin systems, elements of which can be secreted from platelets (Table 1), can have a prominent role (164). As discussed earlier, anucleate, activated platelets can synthesize proteins such as IL-1β, TGF-1β and TF from preformed mRNA (51). Thus when, lipopolysaccharide (LPS) binds to TLR4 and stimulates platelets, it favors sepsis through splicing and translation of IL-1β mRNA (165). Newly synthesized pro-IL-1β is activated by caspase-1 and released bound to MPs or exosomes. It can interact with its platelet receptor IL-1R1 providing an autocrine loop amplifying platelet activation by LPS. Interestingly, Il-1β can also bind to fibrin where it retains its activity while TF favors thrombosis providing another example of a link between thrombosis and innate immunity.

A role for secreted ADP either from platelets and/or tissue cells in systemic inflammation and sepsis, has been confirmed through the use of platelet $P2Y_{12}$ inhibitors in man although both pro- and anti-inflammatory roles have been described (5, 166). Drugs blocking $P2Y_{12}$ dampen down platelet-monocyte and platelet-neutrophil aggregate formation and the release of pro-inflammatory cytokines in response to bacterial endotoxemia. $P2Y_{12}$ inhibition with clopidogrel or its deletion in mice also decreased platelet sequestration in the lungs and lung injury in a mouse model of intra-abdominal sepsis and acute lung injury (167). Reducing P-selectin expression largely brings about these effects. Notwithstanding, platelets also stimulate inflammation through pathways independent of $P2Y_{12}$, for example the TLR4-activated signaling cascade (168). Studies with mice show that platelets may play an important role in host response to Klebsiella pneumosepsis (169). Antibody-induced thrombocytopenia was associated with greater bleeding and a reduced survival of the mice with increased bacterial growth in the lungs. Thrombocytopenia was associated with a dysregulated host response in critically ill sepsis patients; a blood microarray analysis revealed a distinct gene expression pattern in sepsis leading to reduced leukocyte adhesion and diapedesis when the platelet count is low (170). In inflammatory states hepatic TPO production can also be upregulated by IL-6 leading to an overproduction of platelets; platelet clearance in the liver may be part of the acute phase response (22). Intriguingly, a highly inflammatory state can lead to an upregulation of platelet production by direct fragmentation of megakaryocytes (171). In addition, Haas *et al* (172 have defined a normally quiescent hematopoietic stem cell-like progenitor that in situations of acute inflammation becomes primed and allows a rapid replenishment of platelets during inflammatory insult.

Platelets are effectors of injury in a variety of pulmonary disorders (34, 173). Modulation of Wnt/β-catenin signaling by platelet-derived Dickopf-1 (Dkk1) is a major factor in promoting neutrophil trafficking and the inflammatory response in the lungs (174). Dkk1 is an example of the role of a relatively unknown α-granule protein. Platelet secretion of PF4 regulates neutrophil infiltration and lung inflammation in lung damage through stimulating alveolar macrophages to produce CXCL2 in polymicrobial sepsis (175).

8. ADDITIONAL THOUGHTS ON PLATELETS AND INNATE IMMUNITY

Clearly, platelets act as sentinel innate immune cells and by facilitating white blood cell entry into lymph nodes and the spleen help immune surveillance and the clearing of affected tissue of invading pathogens (29, 127). They have also been shown to act as antigen presenting cells. As discussed in the previous Section, platelets participate actively in host defence by binding and capturing pathogens, secreting microbicidal proteins and promoting neutrophils to act similarly, and have a close interplay with the complement system. Intriguingly, platelets phagocytose neutrophil-derived MPs and relocate them so that they become available to cyclooxygenase-1 giving increased TXA_2 production that in turn facilitates endothelial cell expression of ICAM-1 favouring neutrophil recruitment in the vasculature and in particular in the lungs (176). Thrombosis is also recognized as an intravascular effector of innate immunity particularly in small blood vessels (30). Interestingly, mice expressing a mutant form of Fg that cannot support fibrin polymer and clot formation while sustaining platelet aggregation exhibit compromised antimicrobial host defence thereby emphasizing the importance of fibrin (177). As we have discussed in the previous Section, in large vessels platelets stimulate neutrophils to extrude nuclear material with the procoagulant DNA nets forming an essential part of the immune response with fibrin; the nets themselves trapping bacteria. In small vessels it is the expression of TF on leukocytes and their release of nucleosomes that stimulates fibrin formation while released proteases degrade inhibitors of fibrinolysis (30, 153). Platelets are partners in the above processes. We will further illustrate the role of platelets in immunity with reference to three examples of α-granule proteins with specific roles.

8.1. CD40L

A much-studied platelet cytokine is CD40 ligand (CD40L, CD154), first identified on activated helper T cells and a member of the TNF family (88, 178). It binds not only to CD40 on antigen-presenting cells

but also to multiple receptors on a variety of cells, these receptors include the integrins αMβ2, α5β1 and αIIbβ3 thereby also linking it to inflammation and thrombosis. In the immune system, the CD40L/CD40 interaction drives B-cell proliferation and antibody production; it plays a primary role in immunoglobulin class switching and intervenes in autoimmune disorders (179). Platelets constitute the major reservoir for CD40L in blood; present in the α-granule membrane, it is transported to the platelet surface on platelet activation where it becomes available to bind other blood or vascular cells. It participates in inflammation and atherosclerosis by favouring the formation of platelet/leukocyte aggregates and by stimulating interleukin and cytokine production and the release of reactive oxygen species (88, 127, 180). Surface-expressed platelet CD40L is a sheddase substrate for MMPs that release the smaller but still biologically active soluble CD40L (sCD40L). CD40L is also an active surface component of platelet MPs.

8.2. TREM-like transcript-1 (TLT-1)

The triggering receptors expressed on myeloid cells (TREMs) contain a single V-set immunoglobulin (Ig) domain, and are involved in cell activation within the innate immune system with a key role in sepsis. A glycoprotein with significant homology to the TREMs, TLT-1 is exclusive to mouse and human MK lineages where it co-localizes with P-selectin in the α-granule membrane (181). It is translocated to the platelet surface when platelet activation leads to secretion and supports platelet aggregation with Fg among its ligands thereby protecting against bleeding during inflammation. Like CD40L (and P-selectin), TLT-1 can also be the object of cleavage by “sheddase” enzymes to liberate a soluble form that has a regulatory role in sepsis by modulating platelet-neutrophil crosstalk (182).

8.3. High mobility group box 1 (HMGB1)

Recently, HMGB1, a protypical DAMP and a nuclear protein attached to DNA and a regulator of gene expression, is also stored in platelets being translocated to the surface and secreted on platelet activation in inflammatory diseases (183–185). It is also intimately involved in the regulation of DVT (185). Thus, in a mouse model of flow reduction in the inferior vena cava (IVC), a cellular mass formed upstream of the stenosis after 48h. Large amounts of HGMB1, mostly released from platelets, progressively accumulated on the luminal endothelial surface and played a role in the trapping of platelet-leukocyte aggregates via RAGE (receptor for advanced glycation end products) and TLR2/4. Atherosclerosis, thrombosis and inflammation are inseparably linked and in this context HMGB1 appears as a critical player by fine-tuning leukocyte recruitment and activation. Its functionality depends on its redox state with the reduced form acting as a chemoattractant; once oxidized it activates leukocytes. Mice specifically lacking HMGB1 in their platelets have increased bleeding, reduced thrombus formation and platelet aggregation; but they also have less inflammation and organ damage during experimental trauma/hemorrhagic shock and sepsis (183). HMGB1 may also have a role in rheumatoid arthritis being at the crossroads of innate and adaptive immunity; it is another excellent example of a previously unrecognized platelet protein with multiple functions in health and disease. HMGB1 from platelets also plays a key role in the formation of NETs by acting through RAGE on neutrophils and through the induction of autophagy (185–186). In fact, oxidized HMGB1 may be a master regulator of the pro-thrombotic cascade involving platelets and myeloid leukocytes fostering occlusive DVT formation (185). HMGB1 oxidation unleashes its prothrombotic activity and promotes platelet aggregation. Additional monocytes arrive and are activated through RAGE and TLR2 with expression of TF and release of cytokines, an activating cascade that leads to NET formation and obstructive DVT.

9. PERSPECTIVES: OTHER MAJOR DISEASES AND WHAT THE FUTURE HOLDS

This review has been selective and many major pathologies are not mentioned. It is important to briefly comment on how platelets intervene in some of them and how this intervention offers novel therapeutic strategies. An example is cancer for circulating tumor cells may bind to platelets and even aggregate them; an interaction that can protect tumor cells from the immune system and also facilitate metastasis by depositing them in the vasculature through their use of platelet adhesive receptors (e.g. GPIb, integrins, P-selectin) (see 33, 187–188). Release of ADP, ATP and polyphosphates, the expression of P-selectin after platelet activation and the generation of thrombin on the now procoagulant platelet surface may all favor tumor growth within the vessel wall and help tumor stability. The release of a-granule proteins (e.g. VEGF, PDGF, EGF, angiopoietin-1 and TGF-β1) may promote angiogenesis and vascularization of the tumor while autotaxin is a novel platelet secreted enzyme that liberates lysophosphatidylcholine and stimulates tumor cell mobility (188). Intriguingly, platelets from cancer patients have higher levels of VEGF and angiopoietin-1 suggesting either a feedback mechanism or their active uptake (189).

By transporting secretable pools of amyloid-β precursor, a substrate for ADAM10 (α-secretase), and by being activated by amyloid-β in the walls of cerebral vessels leading to thrombus formation and granule release; platelets may actively participate in the progression of Alzheimer’s disease, an

age-related neurodegenerative disorder (190–191). In fact, amyloid-β binds directly to the αIIbβ3 integrin and stimulates release from platelets of ADP and the chaperone protein clusterin, a known player in Alzheimer's disease. The latter promotes the formation of fibrillar amyloid-β aggregates while ADP promoted αIIbβ3 activation and clusterin release thereby accelerating the process. The interaction between P-selectin on activated platelets and PSGL-1 on neutrophils and platelet-derived chemokine heterodimers play a major role in acute lung injury (192). The pro-inflammatory potential of platelets also leads to roles in inflammatory bowel disease (with elevated levels of RANTES), migraine (IL-1 and β-thromboglobulin) and asthma (kallikrein/kinin system) among many examples (see 34, 164). Platelet activation has been postulated to be heavily involved in allergic asthma and $P2Y_{12}$ promotes eosinophilia and the pro-inflammatory action of leukotriene E4 in the airways (193–194).

What does the future hold? Anti-platelet therapy is an increasingly used option in non-hemostatic disorders as for example the use of $P2Y_{12}$ inhibitors to reduce inflammation or blockers of β3 integrins in metastasis and tumor growth (86, 195). An alternative and very promising approach is to genetically modify progenitor cells or MKs so that platelets are produced with α-granules containing new proteins of therapeutic benefit such as FVIII as a treatment for hemophilia or TRAIL for prostrate cancer (196–197). This type of approach may ultimately be used for lifelong therapies in major illnesses including cardiovascular disease, other forms of cancer and Alzheimer's disease but also many others.

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