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TOPICAL REVIEW

Platelets: a critical link between inflammation and microvascular dysfunction

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Abstract Inflammation is an underlying feature of a variety of human diseases. An important manifestation of this pathophysiological response is microvascular dysfunction, which includes the activation of vascular endothelial cells, and circulating leucocytes and platelets. While endothelial cells and leucocytes are widely accepted as critical players in the microvascular alterations induced by inflammation, recent attention has focused on the modulatory role of platelets, which act both as effector and target cells in inflamed microvessels. Evidence is presented to demonstrate the capacity for 'cross-talk' between platelets and other cells (endothelial cells, leucocytes) that contribute to an inflammatory response, and to illustrate the pathophysiological consequences of these interactions of platelets with other cells within the microvasculature.

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Abbreviations AA, arachidonic acid; CD40L, CD40 ligand; EC, endothelial cell; GAG, glycosaminoglycan; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; IL, interleukin; LTB_4, leukotriene B_4; NET, neutrophil extracellular trap; NO, nitric oxide; PAF, platelet-activating factor; PLA, platelet-leucocyte aggregate; PSGL-1, P-selectin glycoprotein ligand-1; RANTES, regulated upon activation, normal T cell expressed; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; TLR, toll-like receptor; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor.

Introduction

It is now well recognized that inflammation is an underlying feature of a variety of diseases that are associated with significant morbidity and mortality. Cancer, sickle cell disease, atherosclerosis, Alzheimer's disease and other conditions have not been traditionally classified as inflammatory diseases; however there is mounting evidence that suggests a role for inflammation in their initiation and/or progression. Inflammatory conditions exhibit several characteristic responses of the microvasculature that allow the affected tissues to mount an inflammatory response, but may also lead to tissue injury and organ dysfunction. For example, impaired blood flow regulation, the recruitment of inflammatory cells, oxidative stress, and enhanced protein and water extravasation are often detected in inflamed tissue. While these responses have been linked to endothelial cell dysfunction caused by the adhesion and activation of

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leucocytes in the microvasculature, there is a large and growing body of evidence that implicates platelets and their activation products in the altered microvascular function that accompanies an inflammatory response (Rhodin *et al.* 2003; Gavins *et al.* 2007; Langer & Gawaz, 2008; Sabrkhany *et al.* 2010). Platelet adhesion and activation not only account for the increased incidence of thrombosis that is associated with acute and chronic inflammatory conditions, but also intensifies, via contact-dependent and -independent mechanisms, the activation of vascular endothelial cells and leucocytes in inflamed microvessels. This review examines the interactions between platelets, vascular endothelial cell (ECs), and leucocytes during inflammation, and addresses how platelets serve as both target and effector cells in the inflammatory response. Evidence for platelet 'cross-talk' in some experimental models of human disease is also summarized.

Crosstalk between platelets, leucocytes and endothelial cells

Platelets as a target in inflammation. As platelets course through the vasculature of inflamed tissue, they are exposed to soluble mediators such as lipid mediators, cytokines and chemokines released by activated leucocytes, ECs and perivascular cells (Fig. 1). These mediators engage with receptors on platelets to elicit an activation response that is characterized by the degranulation of dense granules and/or α-granules, release of platelet products, and mobilization and activation of platelet adhesion molecules. Lipid mediators (e.g. platelet activating factor), cytokines (e.g. interferon-γ (IFN-γ), interleukin-2 (IL-2)) and chemokines (e.g. CXCL12, CCL22) are examples of inflammatory mediators that can activate platelets. The oxidative stress that accompanies inflammation results in phospholipase A2 activation and the generation of

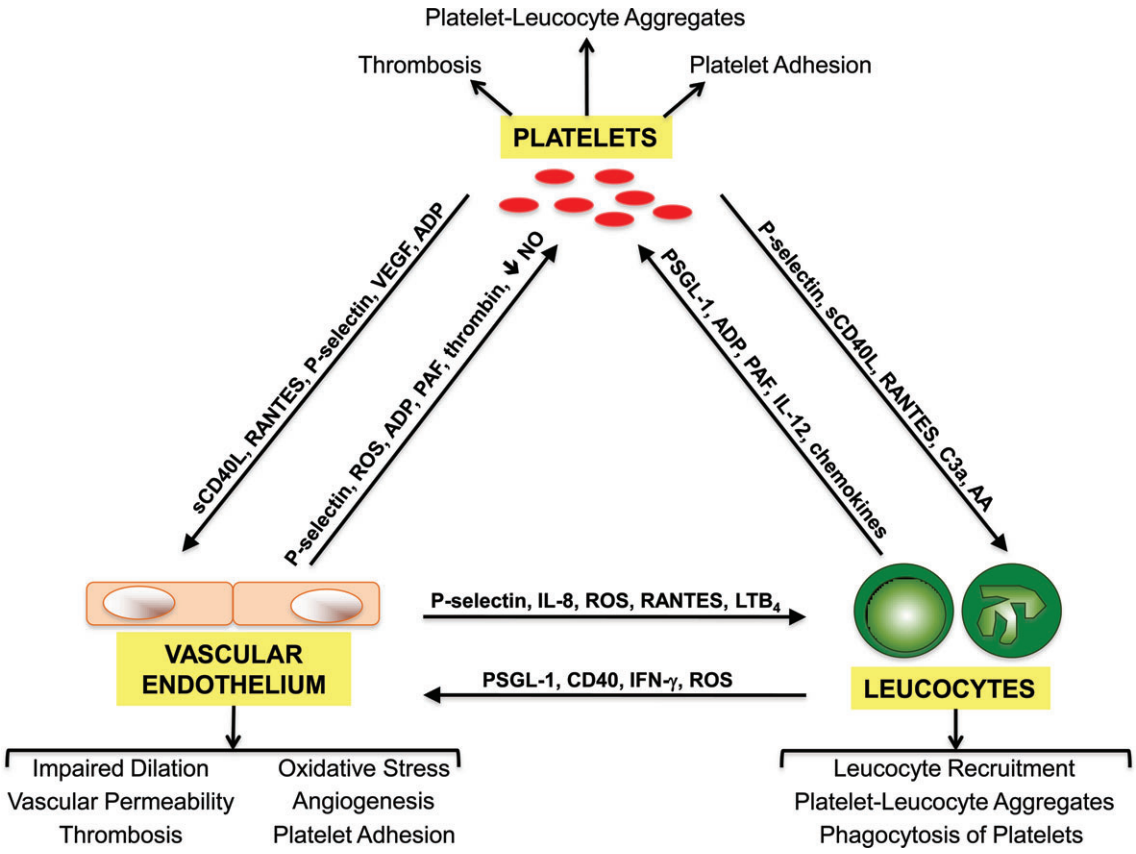

Figure 1. A schematic diagram of the 'cross-talk' between platelets, vascular endothelium and leucocytes in response to stimuli released during inflammatory conditions
The arrows indicate the direction of the communication, and illustrate both the physical interactions (via cell adhesion molecules, such as P-selectin) and soluble factors (e.g. chemokines, cytokines, etc.) that mediate the cell–cell communication via autocrine and paracrine pathways. Some of the pathological changes resulting from this platelet 'cross-talk' are listed below the endothelial cells and leucocytes, and above the platelets. The information portrayed here is discussed throughout the text. AA: arachidonic acid; LTB_4: leukotriene B_4.

PAF and other arachidonic acid metabolites that can also activate platelets. The chemokine receptors CXCR4 and CCR4 are expressed on platelets and when engaged with their chemokine ligands (CXCL12 and CCL22, respectively), platelets express P-selectin and release their own compliment of chemokines and other granular components into extracellular fluid (Gleissner *et al.* 2008). While some cytokines (e.g. IFN-γ) act on platelets to promote the degranulation of dense granules, others (e.g. IL-2) appear to target platelet α-granules (Li, 2008). The accumulation of immune cells in inflamed tissue can also lead to the generation of adenosine diphosphate (ADP), which activates platelets and promotes the degranulation of both dense granules and α-granules. The ecto-ATPase expressed on the surface of lymphocytes can rapidly convert ATP release from platelets and other blood cells to ADP (Stafford *et al.* 2003). In addition to soluble inflammatory mediators, microparticles liberated from the plasma membrane of ECs following cytokine activation can also alter platelet function. It is unclear whether this requires physical contact between the cells or simply the generation of platelet agonists, like thrombin (Dignat-George & Boulanger, 2011), and this may depend on the inflammatory stimulus.

While platelets normally do not physically interact with vascular ECs, activated platelets will bind to the wall of inflamed microvessels by attaching either directly to ECs or to leucocytes that are already adherent on the vessel wall. The absence of platelet adhesion to healthy ECs has been attributed, at least in part, to inhibitory mechanisms involving nitric oxide (NO), prostacyclin, and adenosine that are normally generated by vascular endothelium. Adenosine diphosphate may accumulate in inflamed tissue either as a result of diminished capillary perfusion or due to inhibition of ectonucleotidase CD39, which is expressed on the surface of ECs and circulating immune cells, where it efficiently hydrolyses extracellular ATP and ADP (both of which stimulate platelet adhesion and aggregation) to AMP and ultimately adenosine (which inhibits platelet adhesion and aggregation) (Robson *et al.* 2006). Oxidative stress and proinflammatory cytokines (e.g. tumour necrosis factor-α (TNF-α)) downregulate CD39 thereby favoring the accumulation of ADP and resulting in lower adenosine levels (Robson *et al.* 2006). The excess generation of superoxide in inflamed tissue also results in the inactivation of NO, thereby reducing the levels of this endogenous anti-adhesion molecule. The combined effects of depleting adenosine and NO, with a corresponding accumulation of inflammatory mediators that elicit platelet activation, is likely to lead to an increased expression of adhesion molecules by platelets that enable them to interact with ECs and other circulating blood cells.

According to the classical paradigm of platelet–vessel wall interactions during thrombus formation, vessel injury and exposure of matrix material that underlies the EC layer is required for platelet adhesion. However, it is now recognized that platelets can adhere to the vascular wall in the absence of vessel injury. The EC activation that accompanies inflammation appears to be sufficient, in the presence of platelet activation, to elicit platelet–EC adhesion (May *et al.* 2008). This phenomenon has been demonstrated in both *in vitro* (Li *et al.* 1996; Bombeli *et al.* 1998) and *in vivo* (Massberg *et al.* 1999; Tailor *et al.* 2005) models of inflammation. Different adhesion glycoprotein-ligand pairs have been implicated in the platelet–EC adhesion associated with acute or chronic models of inflammation, including P-selectin (platelet or EC)–P-selectin glycoprotein ligand-1 (PSGL-1) (EC or platelet), GPIb (platelet)–von Willebrand factor (EC), as well as GPIIb/IIIa (platelet)–fibrinogen-intercellular adhesion molecule-1 (ICAM-1) (EC) interactions (Morrell *et al.* 2007; May *et al.* 2008). These molecular interactions allow platelets to roll and firmly adhere to the endothelial cell surface, in a manner similar to leucocyte–EC adhesion.

Platelets as effector cells that amplify the inflammatory response. The binding of platelets to vascular endothelium enables the former to modulate the activation state of the latter (and vice versa). Upon activation, platelets release >300 proteins and small molecules, most of which are biologically active molecules that can influence the function of the vascular wall and circulating immune cells (Coppinger *et al.* 2004). Many of these molecules, including growth factors, cytokines, chemokines, angiogenic factors, ADP/ATP, and coagulation factors, are preformed and stored in dense bodies or α-granules, for release upon platelet activation. Other bioactive molecules are either synthesized by platelets (e.g. thromboxane, reactive oxygen species (ROS), IL-1β) or shed from the cell surface (CD40 ligand (CD40L), P-selectin) upon activation. When platelets adhere to ECs, the close proximity of the two cells enables the platelets to deposit large quantities of chemokines, cytokines and growth factors (e.g. vascular endothelial growth factor; VEGF) that trigger signal transduction pathways that regulate the metabolic, adhesive and proliferative properties of ECs (Siegel-Axel & Gawaz, 2007). Co-incubation of $CD40L^+$ platelets with EC monolayers results in EC activation, increased expression of EC adhesion molecules, including ICAM-1, enhanced production of IL-8 (a neutrophil chemoattractant), and increased leucocyte–EC adhesion (Danese *et al.* 2003*a*). These endothelial responses appear to be dependent on (1) the engagement of platelet CD40L with EC CD40, and (2) secretion of the chemokine, regulated upon activation, normal T cell expressed (RANTES), by platelets (Danese *et al.* 2003*a*), exemplifying this platelet–EC 'cross-talk' (Fig. 1).

CD40L is a membrane glycoprotein in the TNF family that is expressed on platelets and other circulating cells. Platelet CD40L expression is increased during inflammation, as is the plasma concentration of soluble CD40L (sCD40L), 95% of which is shed from the surface of activated platelets (Danese *et al.* 2003*b*). Endothelial expression of CD40 is also increased during inflammatory bowel disease (IBD) (Vowinkel *et al.* 2007*a*). In addition to inducing an inflammatory phenotype in EC, $CD40L^+$ platelets appear to elicit the expression of tissue factor by engaging with CD40 on ECs (Bavendiek *et al.* 2002). Soluble CD40L may also bind platelet glycoprotein GPIIb/IIIa, which helps to stabilize the thrombus and activate more platelets (Prasad *et al.* 2003). Mice that are genetically deficient in either CD40 or CD40L exhibit marked reductions in leucocyte and platelet adhesion in venules inflamed by hypercholesterolaemia (Stokes *et al.* 2009) or colitis (Vowinkel *et al.* 2007*a*). CD40/CD40L also appears to play a role in inflammation-enhanced microvascular thrombosis since CD40L-deficient mice exhibit an attenuated thrombogenic response in arterioles that can be restored to normal after administration of sCD40L (Gavins *et al.* 2011). Finally, there is evidence that supports a role for platelets and CD40L in the impaired endothelium-dependent vasodilatation that accompanies hypercholesterolaemia-induced microvascular inflammation (Stokes *et al.* 2006, 2009).

RANTES, a member of the CC-chemokine family, is produced by a variety of cells, but platelets are considered to be the major source *in vivo* (Aukrust *et al.* 1998). RANTES induces leucocyte chemotaxis by engaging its receptor (CCR5); however the chemokine promotes leucocyte activation/adhesion through an oligomerization-dependent interaction with EC surface glycosaminoglycans (GAGs) (Appay & Rowland-Jones, 2001). The avidity of RANTES for GAGs ensures that blood cell-derived RANTES is concentrated on the EC surface in inflamed or damaged tissue. RANTES has been implicated in the leucocyte adhesion in cerebral venules of mice with experimental autoimmune encephalitis (dos Santos *et al.* 2005) and stroke (Terao *et al.* 2008), as well as in monocyte arrest on cultured ECs (Baltus *et al.* 2003). CXCL7 and CXCL4 are two other platelet-derived chemokines that have been shown to promote leucocyte recruitment (Gleissner *et al.* 2008).

There is mounting evidence that platelets may play an important role in activation of the alternative and classical pathways of complement. Complement activation is associated with the generation of C3a and C5a, which are cytokine-like mediators that promote leucocyte recruitment and amplify the inflammatory response via leucocyte receptors (Del Conde *et al.* 2005; Peerschke *et al.* 2010) and activation of vascular endothelium (Albrecht *et al.* 2004). The production and deposition of complement on/by platelets is dependent on (and proportional to) platelet activation (Peerschke *et al.* 2006). For example, weak activation of platelets by agonists such as ADP supports less complement activation than is induced by thrombin. Furthermore different agonists may induce different pathways of complement. Platelet activation is accompanied by the expression of P-selectin, which is associated with activation of the alternative complement pathway (Del Conde *et al.* 2005). Classical pathway activation is linked to platelet expression of gC1qR (the complement receptor for C1q) (Peerschke *et al.* 2006) and secretion of the primary glycosaminoglycan on platelets, chondroitin sulfate (Hamad *et al.* 2008). Microparticles liberated from activated platelets may also generate/deposit complement components (Peerschke *et al.* 2010). Activated platelets and platelet microparticles exhibit measurable levels of complement (including C1q, C3b, C5b-9) deposition on the platelet cell membrane, which can attract and activate leucocytes by interacting with complement receptors expressed by leucocytes (Del Conde *et al.* 2005; Peerschke *et al.* 2006). Moreover, platelets can be activated by C5b-9 proteins (Sims *et al.* 1988), suggesting a self-propagating loop between complement and platelet activation. It was recently shown that chondroitin sulfate binds C1q (Hamad *et al.* 2008). This appears to amplify binding of immune complexes to the activated platelet, and may help explain how arterial thrombotic events are elevated in the immune-altered state of lupus, which is associated with deposition of C1q and C4d on platelets. Whether complement activation by platelets is in part responsible for the role of platelets in other inflammatory and thrombotic diseases remains unclear, although it is plausible because complement activation has been implicated in many of these, for example atherosclerosis (Manthey *et al.* 2011).

In vitro studies have revealed that, similar to interactions with ECs, leucocytes can roll on and firmly adhere to a layer of immobilized (adherent) platelets (Hammer & Apte, 1992). This results from PSGL-1 on leucocytes binding to P-selectin on activated platelets (rolling), and $\alpha M\beta 2$ (CD11b/CD18) on leucocytes interacting with GPIb and/or fibrinogen on platelets (adhesion). CD40 (leucocyte) interactions with CD40L (platelet) may also contribute to the adhesion response (Smyth *et al.* 2009). *In vivo* studies of the responses of mesenteric (Salter *et al.* 2001), cerebral (Ishikawa *et al.* 2004), and myocardial (Kupatt *et al.* 2002) microvessels to ischaemia–reperfusion support a role for platelets, acting via P-selectin (or GPIIb/IIIa in heart and mesentery), in the modulation of leucocyte recruitment. The opposite can also occur, i.e. adherent neutrophils can also promote the recruitment of platelets via P-selectin:PSGL-1 and/or GPIbα:$\alpha M\beta 2$ binding (Tailor *et al.* 2005; *Vowinkel et al.* 2007*b*). While P-selectin expression is greatly increased in the postischaemic vasculature, this is

not observed in thrombocytopenic animals, suggesting that platelets immobilized on the vessel wall may be responsible for the upregulated P-selectin noted following ischaemia–reperfusion. Bone marrow chimeric mice, produced by the transplantation of bone marrow from P-selectin deficient mice into wild-type recipients (or vice versa), have revealed that the relative roles of endothelial *vs*. platelet P-selectin in the recruitment of leucocytes in venules is model dependent (Tailor *et al.* 2005).

Inflammation is also associated with the binding of platelets to leucocytes that are circulating in the blood (platelet–leucocyte aggregates, PLAs) (Tailor *et al.* 2005). The P-selectin-dependent platelet–leucocyte complexes that are observed in inflamed venules may be a precursor of the free-flowing PLAs that are detected in blood of patients with chronic inflammatory diseases. The pathophysiological significance of platelet–leucocyte adhesion remains unclear. However, there is evidence that pretreatment of platelets with pro-inflammatory cytokines (e.g. IL-1β and TNF-α) leads to enhanced leucocyte–platelet interactions (Todoroki *et al.* 1991), suggesting that leucocytes with attached platelets are primed for adhesion. Furthermore these leucocytes can achieve a more activated state than their platelet-free counterparts. For example, neutrophils and monocytes with attached activated platelets produce more than twice the amount of superoxide than their platelet-free counterparts (Nagata *et al.* 1993), and P-selectin mediated signalling is critical for this response. A recent report indicates that P-selectin dependent formation of PLAs in the circulation initiates signalling pathways in neutrophils that result in the phagocytosis of the attached platelets (Maugeri *et al.* 2009), which may represent a clearance programme to minimize the pathological impact of uncontrolled platelet activation. Another example of the consequences of platelet–leucocyte interactions on cell activation comes from a study showing that PAF generation by the combination of platelets and neutrophils is two times higher than that detected in either cell activated separately. However, this amplification of PAF production does not appear to be dependent on cell–cell adhesion and instead relies on transcellular phospholipid metabolism between the two cells (Coeffier *et al.* 1990).

Platelets also release agents that have the potential to increase or decrease vascular permeability. Exposure of EC monolayers to platelets results in a 'dose-dependent' reduction in albumin permeability, with the greatest inhibition of permeability occurring with the highest platelet concentration (Shepard *et al.* 1989). This barrier protection afforded by platelets is not due to mechanical obstruction of inter-endothelial junctions, but was initially linked to a soluble platelet product, adenosine (Paty *et al.* 1992). Subsequent work implicated an unidentified protein/protein-associated mediator (Patil *et al.* 1997), and sphingosine-1-phosphate (S1P) was later shown to be a prime candidate lipid (Schaphorst *et al.* 2003; Peng *et al.* 2004) (Fig. 2). Studies of pulmonary vascular permeability in thrombocytopenic sheep are consistent with a role for platelets in the maintenance of vascular integrity (Lo *et al.* 1988). However, there are reports to the contrary in inflammation. For example, depletion of circulating platelets has been shown to blunt the increased vascular permeability and leucocyte recruitment noted after aseptic cutaneous wounding (Kim *et al.* 2009). These opposing

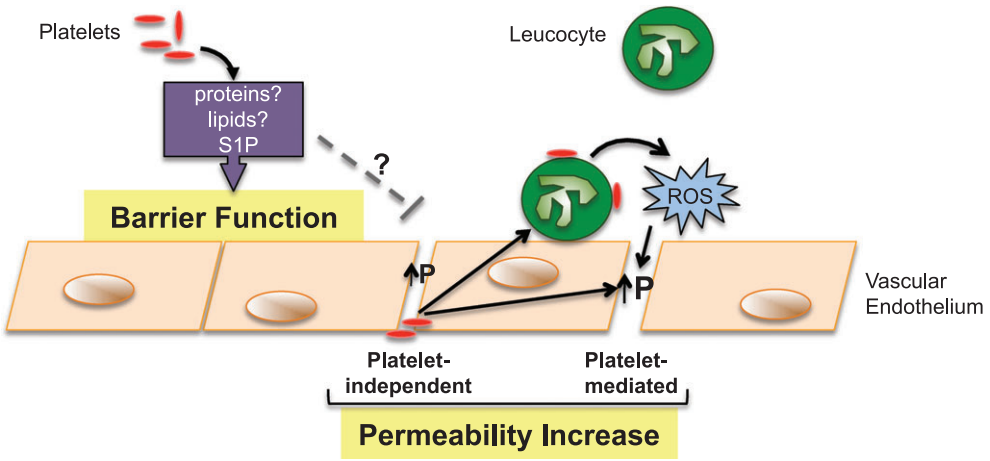

Figure 2. A proposed schematic of the opposing roles of platelets in endothelial barrier function. Platelets maintain normal barrier function through the release of soluble factors, most likely proteins, or lipid mediators such as sphingosine-1-phosphate (S1P). However under inflammatory conditions, this protective function of platelets may be overcome. Vascular permeability (P) may be initially increased in a platelet-independent manner, allowing platelet accumulation on the subendothelial matrix. These platelets may become activated and release factors that act directly, or indirectly via recruitment of leucocytes, to further disrupt the barrier function. This latter pathway is mediated via a burst of ROS generation from the leucocytes

roles for platelets in endothelial barrier function likely reflect the diverse mediators released by these cells and the intensity of the platelet activation response elicited in different models of inflammation.

It is now recognized that the leucocyte-mediated endothelial barrier dysfunction that accompanies an inflammatory response is dependent on the generation of ROS (by leucocytes), and that recruited and circulating leucocytes that are submaximally activated will not promote vascular leak due to inadequate ROS generation (Zhu *et al.* 2005; Zhu & He, 2006). Therefore, the role for platelets as mediators of endothelial barrier dysfunction may also depend on whether these cells can activate adherent or circulating leucocytes sufficiently to yield the required level of ROS generation. In some models, an early platelet-independent increase in vascular permeability may result in platelet attachment to the subendothelial matrix and subsequent activation. These platelets may release mediators that directly increase permeability, or the platelets could promote the recruitment of leucocytes that are sufficiently stimulated by platelets to elicit an oxidative burst and increase vascular permeability (Fig. 2). Such a scenario is consistent with the findings reported by He *et al.* (2006). However, there are also reports suggesting that platelets may exert opposing actions on inflammation and vascular permeability in the tumour microvaculature. Depletion of platelets reduces tumour metastasis presumably by decreasing inflammation, while enhancing the efficacy of chemotherapy by enhancing vascular permeability (Demers *et al.* 2011). While it may be therapeutically advantageous to transiently increase the vascular leakiness in tumours, such an approach may lead to serious consequences in tissue such as the lung. Therapeutically, perhaps a balance can be struck between enhancing the protective properties of platelets (as was shown for aprotinin treatment against bradykinin-induced oedema; O'Brien *et al.* 1997), and inhibiting the platelet-promoting pathways of vascular leak (including their cellular targets) in order to achieve maximal protection against platelet-mediated vascular dysfunction.

Angiogenesis is another vascular response to inflammation that can be influenced by platelets. Activated platelets release a variety of angiogenic growth factors, including VEGF, platelet derived growth factor and fibroblast growth factor. *In vitro* studies have revealed that both platelets and platelet microparticles stimulate EC proliferation and promote the formation of capillary-like structures (Pipili-Synetos *et al.* 1998). Thrombocytopenia is associated with reduced angiogenic responses to inflammation and/or tissue injury (Kisucka *et al.* 2006). Platelets appear to preferentially adhere in angiogenic blood vessels and interference with this adhesion process blunts blood vessel proliferation and induces haemorrhage from the angiogenic vessels (Kisucka *et al.* 2006). The enhanced angiogenic response elicited by erythropoietin treatment in ischaemic tissue also appears to be dependent on platelet adhesion via P-selectin (Kato *et al.* 2010). The identity of the platelet product that mediates the adhesion-dependent pro-angiogenic effect in these experimental models remains unknown.

Evidence for platelet 'crosstalk' in different diseases

From the sections above it is clear that platelets have the potential, through a variety of different mechanisms, to contribute to the pathogenesis of many diseases that have an inflammatory component, some of which were discussed above. Here we provide further discussion of a few of these diseases.

Cardiovascular diseases. Cardiovascular disease is the leading cause of death worldwide. Platelets contribute to the inflammatory process underlying large vessel disease through direct interactions with leucocytes and/or the vessel wall (dependent on P-selectin (Huo *et al.* 2003), GP1a, GPIIbIIIa (Massberg *et al.* 2002), and CD40L (Lievens *et al.* 2010)), and by depositing chemoattractants such as RANTES on the vessel wall (Huo *et al.* 2003). Activated platelets injected into atherogenic mice interact with the diseased area of the vessel in either 'leucocyte-free' form, or bound to leucocytes (primarily monocytes) (Lievens *et al.* 2010). Thus it is unclear whether the phagocytosis of injected activated platelets by neutrophils that is observed in normal mice occurs during the development of atherosclerosis. Nonetheless, there is evidence in humans that such phagocytosis occurs following a cardiac event (Maugeri *et al.* 2009). The entire microvasculature, including the vasa vasorum, is exposed to the same cardiovascular risk factors as large vessels, and many of the same mechanisms involved in lesion development underlie the preceding microvascular endothelial dysfunction which is characterized by impaired vasodilatation in arterioles, leucocyte and platelet recruitment in postcapillary venules, increased oxidative stress, and barrier dysfunction. Early during hypercholesterolaemia, neutrophils mediate the platelet recruitment while platelets, acting via P-selectin, reciprocate to recruit more leucocytes (Stokes *et al.* 2006). These cells also mediate the arteriolar dysfunction, most likely through soluble factors released into the blood and/or from leucocytes and platelets recruited to nearby venules (Kim *et al.* 2007). CD40L expressed by T lymphocytes (Stokes *et al.* 2009), and IFN-γ derived from these cells (Stokes *et al.* 2007), participate in this platelet-dependent inflammatory response, indicating a role for the immune system in the platelet-dependent responses in the early phases of hypercholesterolaemia. These factors are also involved in the

NADPH oxidase-dependent oxidative stress induced in arterioles and venules by hypercholesterolaemia. Such a complex network is also in evidence in stroke, where T cells and IFN-γ (primarily from a non-T cell source) mediate not only the leucocyte and platelet recruitment in cerebral postcapillary venules, but also the tissue injury (Yilmaz *et al.* 2006). RANTES, likely to be derived from platelets, also participates in this brain injury response (Terao *et al.* 2008), supporting the possibility of platelet 'cross-talk' with immune cells and the vascular wall in ischaemic stroke. The microvascular responses to cardiovascular risk factors may predispose organs to worse injury following an ischaemic insult precipitated by thrombus formation or the lodging of emboli in major vessels of the heart or brain. There is also some evidence that the microvasculature is more vulnerable to thrombus development in the presence of risk factors such as hypertension (Senchenkova *et al.* 2010) and hypercholesterolaemia (Broeders *et al.* 2002). It remains unclear whether this enhanced vulnerability to microvascular thrombosis involves an altered communication between platelets and other cells involved in thrombogenesis.

Sickle cell disease. Sickle cell disease is a genetic disorder characterized by abnormal haemoglobin, which distorts the shape of red blood cells. These cells become lodged in the microvasculature leading to a painful sickle cell crisis. This vaso-occlusive state involves interactions between the leading edge of neutrophils (that are interacting with the vessel wall) and sickle red blood cells via the adhesion molecule $\alpha M\beta 2$ (Hidalgo *et al.* 2009), supporting an inflammatory component in sickle cell disease. Others have shown that the elevated leucocyte recruitment in sickle cell transgenic mice is accompanied by higher platelet adhesion in postcapillary venules, and both of these are exacerbated by a hypoxic insult, when compared to control mice (Wood *et al.* 2004*b*). Endothelial P-selectin largely accounted for the recruitment of both leucocytes and platelets; however, the higher constitutitve expression of P-selectin in sickle cell transgenic mice could be attributed to platelet-associated P-selectin (Wood *et al.* 2004*a*), which could serve to promulgate the inflammatory response by interacting with PSGL-1 on leucocytes. In human disease, there is evidence of platelet activation and a corresponding increase in plasma sCD40L, which is likely to result from platelet shedding. The elevated platelet activation state in sickle cell patients may result from excess thrombin generation, which is supported by increased plasma thrombin levels. The clinical relevance of these findings is exemplified by the results of a phase 1 trial wherein the GPIIbIIIa antagonist eptifibatide was shown to be effective in reducing circulating levels of inflammatory mediators and sCD40L, and in attenuating platelet aggregation in sickle cell patients (Lee *et al.* 2007).

Inflammatory bowel disease. The two major forms of IBD are Crohn's disease and ulcerative colitis. Platelets from these patients exhibit enhanced homotypic and heterotypic aggregation responses (Andoh *et al.* 2006; Pamuk *et al.* 2006), and the active phase of IBD is associated with thrombocytosis. The activated platelets from patients with ulcerative colitis enhance the capacity of neutrophils to produce ROS, and this requires P-selectin-dependent adhesion (Suzuki *et al.* 2001). Both platelets and neutrophils are recruited to postcapillary venules of inflamed colons, with each recruitment process influencing the other (Vowinkel *et al.* 2007*b*). A majority of the platelets that accumulate in venules do so via interactions with adherent leucocytes, presumably via platelet P-selectin–leucocyte PSGL-1–endothelial P-selectin (based on studies employing immunoblockade or genetically deficient mice) (Mori *et al.* 2005; Vowinkel *et al.* 2007*b*). P-selectin also appears to mediate the enhanced vascular permeability observed during colitis, suggesting the cell–cell interactions supported by P-selectin are critical to the subsequent endothelial barrier dysfunction. Both CD40 and CD40L have been implicated in the recruitment of platelets and leucocytes in experimental colitis (Vowinkel *et al.* 2007*a*). Given that platelets from colitic patients expressed elevated levels of CD40L, and their circulating levels of sCD40L, derived primarily from platelets, is raised (Danese *et al.* 2003*b*), it is plausible that both platelet-associated CD40L and platelet-derived soluble CD40L interact with vascular endothelium to induce an inflammatory phenotype. Interestingly, it appears that the interaction between platelets and neutrophils does not end at the vessel wall, because platelets have been observed to infiltrate the colon interstitium and move into the gut lumen along with neutrophils in IBD patients. Whether the extravasation of platelets potentiates the inflammatory response remains unclear, although there is evidence suggesting that this process may exacerbate the fluid secretion and diarrhoea associated with IBD (Weissmuller *et al.* 2008).

Patients with IBD are at increased risk for thromboembolism, which is one of the causes of death in this population. Thrombi can form both within the bowel and in extra-intestinal tissues. While it is difficult to know whether the inflammation detected in intestinal venules is linked to the extra-intestinal thrombosis, some mediators, such as CD40L, have been linked to both processes, suggesting that platelet-derived sCD40L generated in the gut enters the bloodstream to mediate thrombosis in distant vascular beds (Gavins *et al.* 2011). The cells/stimuli that are responsible for promoting the platelet release of sCD40L is unclear, but it is likely to be the result of an interaction (and communication) between platelets and other cell types. Although inflammatory cytokines levels are elevated in many disease states, little is known about their role in thrombosis. However, recent work has implicated

both IL-1β and TNF-α in the accelerated thrombosis that occurs in extra-intestinal tissues during experimental IBD (Yoshida *et al.* 2011), supporting a further link between inflammation and platelets in this group of diseases.

Sepsis. During sepsis, there is an initial activation of platelets and heightened coagulation state, which can progress towards reduced platelets counts and exhaustion of the coagulation system with severe sepsis (Mavrommatis *et al.* 2000). While a role for platelets in sepsis has been proposed, their overall contribution is difficult to define because platelets can have opposing roles in this condition and different strains of bacteria may activate the platelets, while others inhibit it. As one of the first responders to invading bacteria, platelets bind the bacteria primarily through toll-like receptors (TLRs) or a plasma protein bridge to GPIIb-IIIa or GPIbα, and are therefore poised to direct the ensuing immune response (Cox *et al.* 2011). TLR activation on platelets results in an outpouring of TNF-α (Leslie, 2010) and it promotes the binding of platelets to neutrophils. The neutrophils respond by releasing DNA fibres that are composed of histones and proteases. The sticky DNA fibres, called neutrophil extracellular traps (NETs), ensnare and kill bacteria that gain access to blood (Clark *et al.* 2007). In addition, sepsis is associated with enhanced thrombosis (Patel *et al.* 2010) and NETs may also provide a stimulus and scaffold for the formation of a red blood cell rich thrombus (Fuchs *et al.*), further supporting the link between inflammatory and platelet responses.

During bacterial infection, neutrophils are activated in such a manner as to guide them to tissues that have not been exposed to the bacteria, and this inappropriate neutrophil accumulation in tissues begets the multi-organ injury that leads to the high mortality rate in septic patients. Neutrophils have been shown to promote platelet recruitment in postcapillary venules of the small bowel and liver (Cerwinka *et al.* 2003; Singer *et al.* 2006) of mice with endotoxaemia, via a ROS-mediated pathway. Sepsis is also associated with capillary bed plugging that has been linked to platelet adhesion to capillary endothelium via a mechanism that is dependent on P-selectin expression, activation of coagulation, and the production of ROS by NADPH oxidase (Tyml, 2011). This intensive sequestration of platelets in capillaries may contribute to the low platelet count that occurs during severe sepsis. There is also evidence that lymphocytes contribute to the leucocyte and platelet adhesion in postcapillary venules during the early stages of sepsis, but may be protective against the tissue injury (Singer *et al.* 2008), underlining the different roles each cell type may play in this systemic condition. In contrast, while neutrophil depletion conferred protection against the tissue inflammation and oedema in a murine model of *Streptococcus pyrogenes*-induced sepsis, induction of thrombocytopenia had no effect despite evidence of microthrombosis (Zhang *et al.* 2011). Therefore caution must be taken when extrapolating a role for platelets in sepsis induced by one bacterial pathogen to other models of infection.

Cancer. The tumour environment is one of inflammation, in which the tumour cells release chemokines and cytokines that attract inflammatory cells, which in turn release factors that the tumour cell uses to survive, proliferate and metastasize (Coussens & Werb, 2002). Platelets too have been implicated, with thrombocytosis common in a number of cancers, and platelet count inversely correlated with survival (Bambace & Holmes, 2011). Tumour cells secrete factors such as ADP and thrombin that can activate platelets. Both platelets and leucocytes can form aggregates with tumour cells that can facilitate tumour cell attachment to vessels and invasion of the tissue. The selectins, including P-selectin on both ECs and platelets (Kim *et al.* 1998), and the $\alpha4\beta1$ integrin on myeloid cells (Schmid *et al.* 2011) have been implicated in metastasis. An angiogenic response is necessary to supply the growing tumour mass with oxygen and nutrients and to offer vascular access for further metastasis. Platelets and inflammatory leucocytes can release factors that destabilize existing vessels, promote capillary sprout formation and enhance EC proliferation. In support of these concepts, platelet depletion has been shown to inhibit pulmonary metastasis in a mouse model, and this could be reversed by administration of platelets, but not platelets that had been preincubated with a GPIIbIIIa antibody (Nierodzik *et al.* 1995). Therapeutic thrombocytopenia may also be beneficial if applied before chemotherapy since platelets normally act to maintain the endothelial barrier function in tumour microvessels, and depleting platelets would enhance vascular leak, thereby enhancing the delivery of chemotherapeutic drugs into the tumour (Demers *et al.* 2011). Several clinical studies also suggest that anti-platelet therapies may be of benefit as an adjuvant to current anti-cancer treatments (Bambace & Holmes, 2011). Inasmuch as platelets potentiate inflammatory responses and minimize tumour vascular permeability, such an approach may have pluripotent effects. However, a role for platelets cannot be globally applied to all cancers, and a recent study was unable to extrapolate promising *in vitro* findings to an *in vivo* murine model of glioblastoma (Brockmann *et al.* 2011). While these conflicting findings may relate to the specific type and/or stage of cancer, work is ongoing to determine the efficacy of targeting platelets to treat the inflammatory and metastatic components of this disease.

Conclusion

While platelets are best known as primary mediators of haemostasis, there is growing recognition that these anuclear cellular fragments may play an equally important role in inflammation. The widely held view that haemostasis and inflammation are intimately linked pathophysiological processes can, in large part, be explained by the capacity of activated platelets to avidly bind to, and communicate with, other platelets as well as ECs and leucocytes. A consequence of this 'cross-talk' between platelets and other cells that participate in the inflammatory response is a more robust reaction of the microvasculature and other tissue components to inflammatory stimuli. The capacity of platelets to both respond and contribute to inflammatory signals places this cell population at the centre of different pathophysiological processes that underlie the tissue injury and organ dysfunction that are associated with a variety of diseases characterized by high morbidity and mortality. The rapidly expanding knowledge about how platelets can function to mediate haemostasis and modulate inflammation may lead to novel and effective therapeutic strategies for the long and growing list of pathological conditions that involve both thrombosis and inflammation. However, consideration must be given to the beneficial actions of platelets that are related to the release of factors such as adenosine and prostacyclin, and the potential negative consequences of depressing platelet function. Similarly, blocking steps in the thrombosis pathway may be deleterious in some conditions. Therapeutic advances are likely to result from a continued effort to reveal the mechanisms that underlie the platelet's capacity to communicate with and excite other circulating blood cells and cellular components of the vascular wall, and how intervening in these pathways alters the protective properties of platelets.

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