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Platelets Are Not Just for Clots

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ABSTRACT

Although the role of platelets as central mediators of hemostasis and thrombosis has been the primary focus of research into platelet biology for more than a century, over the last decade, nonhemostatic functions of platelets have been increasingly defined. As such, a large body of experimental evidence now exists, which places the platelet as a key player in mediating a diverse range of immune, inflammatory, and malignant disease processes. This review outlines the central mechanisms that underpin the nonhemostatic role of platelets and provides a summary of evidence demonstrating a role for platelets in mediating selected inflammatory, immune, and malignant disease processes.

Contents

The fundamental role of platelets in hemostasis was first identified by the Italian pathologist and pioneering medical researcher Bizzozzero in 1882 [144]. Subsequently, for more than 120 years, science has focused on the central role of platelets as the key cellular components mediating hemostasis and, latterly, as pivotal instigators of pathological thrombosis. These classical roles of platelets in mediating thrombosis and hemostasis are now well accepted. However, over the past 2 decades, an ever-increasing literature has evolved linking platelets with biological responses beyond hemostasis and thrombosis. Emerging evidence in the field of platelet biology supports a major proinflammatory function for platelets, linked to host defence and a variety of autoimmune, inflammatory, and malignant diseases processes. The weight of this evidence suggests that platelets may be as important for host defenses and pathological inflammatory and malignant diseases as they are for hemostasis and thrombosis. The purpose of this review is to therefore

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provide a brief overview of the classical role of platelets followed by a discussion of the interactions between platelets and the immune system, and the emerging role for platelets in disorders beyond hemostasis.

The Classical Role of Platelets

The hemostatic function of platelets is of fundamental importance in order to maintain the integrity of a closed, high-pressure circulatory system [1]. Ordinarily platelets circulate in a quiescent state, near the endothelial cells lining the blood vessels without forming stable adhesions. However, after breaches of the vasculature, a number of highly reactive subendothelial matrix proteins become exposed including von Willebrand factor (VWF), collagen, fibrinogen, and fibronection. Initial platelet adhesion is mediated principally by platelet surface receptors interacting with their cognate ligands. Initial platelet adhesive interactions are mediated by platelet surface glycoproteins (GPs) including GPVI and GPIbα interacting with endothelial bound collagen and VWF, respectively [2,3]. These receptor-ligand interactions initiate a cascade of intracellular responses resulting in amplification of platelet activation responses through the secretion of soluble agonists including thromboxane A_2 (TXA_2) and ADP, which act in concert with the engaged platelet receptors to mobilize intracellular Ca^{2+}, which instigates platelet shape change, degranulation, and up-regulation of adhesive function of integrin $\alpha_{IIb}\beta_3$. Integrin $\alpha_{IIb}\beta_3$, in its active conformation, can then bind fibrinogen with high affinity allowing a hemeostatic platelet aggregate or plug to form [4]. The fundamental role of VWF, GPIbα, and $\alpha_{IIb}\beta_3$ in normal hemostatic responses is evidenced by the bleeding diathesis observed in patients with von Willebrand disease, Bernard-Soulier syndrome [5], and Glanzmann thrombasthenia [6], who have quantitative or qualitative abnormalities of VWF, GPIbα, and $\alpha_{IIb}\beta_3$ respectively. The more detailed aspects of the hemostatic role of platelets have been extensively reviewed elsewhere [3,7,8].

Evolving Concepts in Platelet Biology—Linking Hemostasis, Inflammation, And Immunity

Platelets are the second most abundant cell type in the circulation after red blood cells, with an average of 1 trillion platelets present at any one time [3,9]. In addition to their vast number, the small size of platelets aids in their role as guardians of the vasculature and sentinels for infection. The average discoid platelet is approximately 2 μm in diameter. Owing to this small size, under laminar flow conditions, platelets are "pushed" to the periphery by larger erythrocytes and leukocytes, forcing them into close contact with the vessel wall [9]. This allows for the rapid detection of any breaches in the endothelium in addition to sensing any invading pathogens.

Platelets contain 3 principal types of cytoplasmic granules, including α-granules, dense granules, and lysosymes. These granules possess a vast array of proteins that have autocrine and paracrine functions and include adhesive proteins, coagulation factors, chemokines, cytokines, growth factors, proteoglycans, immunoglobulins, and proteases and protease inhibitors. In addition to prestored mediators, recent evidence from proteomic studies suggests that platelets have an extensive transcriptome and proteome [10,11]. Although platelets lack a nucleus and hence genomic DNA, they do contain messenger RNA (mRNA) and all the required translational machinery required for protein synthesis. Recent evidence suggests that after platelet activation by potent agonists such as thrombin, the platelet releasate contains in excess of 300 proteins [11,12]. Consequently, when platelets become activated, they degranulate releasing a vast array of cytokines and chemokines that play a key role in modulating immune responses. Thus, conceptually, the immune response to tissue injury is guided, in part, by the thrombotic response by way of the numerous proteins stored within platelet dense and α-granules (see Table). Hence, it is becoming increasingly recognized that platelets may be as important for host immunity and inflammation as they are for hemostasis. Below we will detail the mechanisms by which platelets may act as inflammatory and immune mediators followed by a summary of the current evidence demonstrating a central role for platelets in mediating the pathogenesis of a diverse range of clinical conditions.

Platelets as Sentinels in Antimicrobial Surveillance and Response to Invading Pathogens

Platelets play an essential role in pathogen surveillance, containment, and wound healing [13]. Platelets are the first and most abundant cell type to accumulate at sites of intravascular infection [14], where they likely play a central role in surveillance and recognition of microbial invasion [15]. In addition, platelets are able to orchestrate complex immune and inflammatory events through a combination of direct cell-cell contacts as well as through the release of a vast arsenal of bioactive molecules (see above and below) that facilitate "information transfer" with immune effector cells [15]. Platelets have been shown to have a role in aiding the immune defense against infections by bacteria, parasites, viruses, fungi, protozoa, and spirochetes [15]. Furthermore, platelet granules contain platelet microbicidal proteins and peptides including the classical chemokines (kinocidins and thrombocidins), platelet factor 4 (PF4), CTAP-III, regulated on activation normal T expressed and secreted (RANTES), fibrinopeptide B, and thymosin β-4, which act on a broad range of pathogens, including some strains of *Staphylococcus aureus* (*S aureus*), viridans streptococci, and *Candida albicans* [16]. In addition, platelet microbicidal proteins have also been shown to potentiate the effect of antibiotic therapies [15].

Activated platelets have been shown by Youssefian et al [17] to engulf both HIV particles and *S aureus* in vacuoles that became a prominent site of α-granule release. These studies suggest that activated platelets expressing P-selectin may assist the phagocytosis and limit the spread of microorganisms by guiding leukocytes to areas of tissue injury where invading microorganisms are trapped by platelets. However, it is conceivable that the HIV virus and the bacteria use platelet engulfment to evade the immune system, as neither killing nor digestion of microorganisms has been demonstrated within platelets. The interaction between platelets and *S aureus* has been implicated in the pathogenesis of infective endocarditis [16]. In protozoal infection by *Toxoplasma gondii*, platelets have been observed to kill the parasites via a mechanism involving TXA_2 synthesis [18]. In malarial infections, platelets are able to kill *Plasmodium falciparum*–infected human red cells in vitro, and in a murine malaria model, platelet depletion or aspirin treatment reduced survival of *P falciparum* parasites [19]. This, however, presents a biological paradox as platelets are implicated in the pathogenesis of cerebral malaria, a condition associated with a high mortality. Platelets may orchestrate this deleterious effect either via binding to and activating the endothelium, or through the formation of aggregates between parasitised red cells and platelets, which can obstruct blood vessels [20–23].

Platelet Immunoreceptors and Toll-Like Receptors

Many of the immunologic functions of platelets involve interactions with platelet immunoreceptors including members of the Fc receptor (FcR) and toll-like receptor (TLR) families.

Platelets express FcRs, which are able to recognize IgG, IgE, and IgA immunoglobulin subclasses on their surface. The FcRs expressed by human platelets include FcεRII, FcγRIIA, and FcαRI [24]. FcγRIIA has been implicated in the development of heparin-induced thrombocytopenia [25] as well as the pathogenesis of bacterial endocarditis where platelet activation occurs via parallel binding between FcγRIIA and IgG antibodies directed against the bacteria and fibrinogen binding to $\alpha_{IIb}\beta_{3[16]}$. The platelet collagen receptor GPVI is also a member of the Ig superfamily. GPVI forms a complex with the immunoreceptor tyrosine activation motif–containing FcRγ, which is critical for GPVI signaling [24,26]. An additional immunoreceptor tyrosine activation motif

Table
Key thromboinflammatory modulators released by platelets [15,64,69,85,139–143]

Localization within the platelet	Released molecule	Target cell	Function
Dense granule	• Histamine	Endothelial cells, monocytes, neutrophils, NK cells, T and B cells and eosinophils	Increased endothelial permeability, expression of adhesive ligands on the endothelial surface and endothelial cytokine and chemokine release Activation of leukocytes—proinflammatory
	• 5HT	Monocytes and T cells	Monocyte and T-cell activation—proinflammatory
α-Granules			
• Adhesion receptors	• $\alpha_{IIb}\beta_{III}$	Platelet	Platelet aggregation, platelet-endothelial, and platelet-leukocyte adhesion
	• GPIbα	Platelet	Platelet adhesion under shear. Platelet-leukocyte interaction via GPIbα-Mac-1 binding
• Adhesive ligands	• P-selectin	Platelets, endothelial cell, and leukocytes—PSGL-1	Adhesion of leukocytes to platelets and endothelial cells
• Chemokines	• CXCL1–GRO-α	Neutrophils and monocytes	Induces neutrophil chemotaxis and monocyte adhesion under flow
	• CXCL4-PF4	Neutrophils and monocytes	Synergizes with other cytokines, leukocyte adhesion, and monocyte differentiation
	• CXCL5–ENA-78	Neutrophils	Induces neutrophil chemotaxis
	• CXCL7–NAP-2	Neutrophils	Enhances neutrophil and monocyte adhesion and neutrophil transendothelial migration
	• CXCL8–IL-8	Neutrophils, T cells, and basophils	Induces chemotaxis and neutrophil activation
	• CCL2–MCP-1	Monocytes and T cells	Monocyte chemotaxis and infiltration and T-cell production of IL-4
	• CCL3–MIP-1α	Monocytes, NK cells, T and B cells, eosinophils, and basophils	Induces chemotaxis and activation
	• CCL5-RANTES	Endothelium/monocytes	Promotes monocytes adhesion to the endothelium and chemokine synthesis
• Growth factors	• PDGF	Vascular smooth muscle cells, monocytes, macrophages, T cells, and B cells	Promotes wound. Stimulates vascular smooth muscle cell migration and proliferation associated with intimal hyperplasia
	• TGF-β	Endothelial cells, monocytes, lymphocytes, NK cells, and neutrophils	Affects multiple regulatory pathways to influence chemotaxis, activation, chemokine release, and cell survival
and• Synthesized cytokines	• IL-1β	Endothelial cells	Up-regulation of leukocyte adhesion molecules and stimulation of endothelial release of proinflammatory cytokines
α-Granule and platelet membrane			
	• CD40 ligand	Endothelial cell CD40	Up-regulation of leukocyte adhesion molecules and stimulation of endothelial release of proinflammatory cytokines. B cell-class switching
• Proinflammatory lipids	• TXA_2	Platelets, macrophages, T cells	Platelet activation, promotes inflammation
	• Leukotriene B_4	Leukocytes—BLT-1 and BLT-2	Chemoattractant and promotion of monocyte differentiation into foam cells and neutrophil activation
	• PAF	Platelet/Leukocytes	Platelet agonist and promotes neutrophil adhesion

5HT, 5 hydroxy-tyramine; TGF-β, transforming growth factor β.

expressed on the surface of platelets is C-type lectin-like receptor 2, which induces potent platelet activation signals via Syk kinase-dependent signaling pathways [27,28]. Activation of platelets through C-type lectin-like receptor 2 binding to its cognate ligand podoplanin facilitates blood/lymphatic vessel separation [29]. This interaction has also been implicated in tumor metastasis [27].

Toll-like receptors are a family of evolutionarily conserved germ-line encoded pattern recognition receptors that recognize conserved molecular motifs expressed by pathogens referred to as pathogen-associated molecular patterns (PAMPs) [30]. Toll-like receptors are divided into subfamilies according to the type of PAMPs they recognize, which include lipoproteins, lipids, proteins, and nucleic acids derived from a wide range of microbes such as bacteria, viruses, parasites, and fungi [31]. Toll-like receptors are not only expressed by professional phagocytic cells (neutrophils, dendritic cells, and macrophages) but are also expressed by platelets [9]. Studies have demonstrated that both murine and human platelets express TLRs1-9, many of which are functional [9] and have been implicated in protective responses to invading pathogens as well as in pathologic immune responses [32–34]. A well-characterized and studied TLR family member is TLR4, which recognizes bacterial lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria, which is a potent immunostimulant that can cause septic shock [31]. Lipopolysaccharide-induced TLR4 signaling in murine models induces platelet adhesion to fibrinogen and platelet sequestration in the microvasculature of the lung, liver, and spleen [35] as well as thrombocytopenia and release of the potent proinflammatory cytokine tissue necrosis factor α (TNF-α) [33]. In addition, recognition of LPS by platelet TLR4 has been shown to induce platelet-neutrophil binding and subsequent robust neutrophil activation resulting in the formation of neutrophil extracellular traps (NETs) [36]. As discussed below, although NETs may represent a potential bacterial trapping mechanism in severe sepsis, exaggerated NETs formation is deleterious to the host. Expression of functional TLR2 has also been demonstrated in platelets [37]. Platelet TLR2 stimulation by bacteria or viral pathogens via the phosphoinositide 3-kinase signaling pathway is able to directly activate both the prothrombotic and proinflammatory platelet responses [37–39] and thus may exacerbate the thrombotic complications of sepsis. Recently, Podrez and colleagues [40] demonstrated that platelet TLR9 activation is an important factor linking platelet activation with thrombosis, oxidative stress, and innate immunity. Interestingly, platelet TLR9 is uniquely distributed to the electron-dense tubular system–related compartment of the platelet, which has been labeled the "T granule" [41]. In T granules, TLR9 is associated with either VAMP 7 or VAMP 8, its association with the latter is proposed to increase TLR9 surface expression via VAMP 8–mediated T-granule fusion with the open canalicular system [41]. In the model proposed by Thon and colleagues [41], platelets are primed to express TLR9 on their surface during activation, which subsequently facilitates secondary activation through PAMPs expressed on invading pathogens. Therefore, platelet-derived TLRs may be as important in orchestrating platelet thrombotic and inflammatory as they are in alerting the innate immune system to pathogen invasion.

Platelet CD40 Ligand, a Central Modulator of the Immune Response

CD40 ligand (CD40L) is a transmembrane protein with structural homology to TNF-α [42] and is an important bioactive modulator of adaptive immunity. Although CD40L was first identified on the surface

of activated CD4$^+$ T cells, it has subsequently been demonstrated that platelets are a rich source of CD40L [43], with greater than 95% of circulating CD40L being of platelet origin [44]. CD40 ligand interacts with its cognate receptor CD40 expressed on numerous cell types including smooth muscle cells, endothelial cells, fibroblasts, dendritic cells, leukocytes, and platelets [42,45]. Through CD40L-dependent mechanisms, platelets are able to activate dendritic cells, resulting in release of proinflammatory cytokines and chemokines as well as activation of naïve T cells, which are fundamental to the establishment of cellular immune responses [9,46]. Within the humoral immune system, CD40L plays a critical role by supporting T-cell–dependent B-cell differentiation and immunoglobulin isotype switching [47]. The importance of this role of CD40L in humoral immunity is evidenced by the severe immunodeficiency state characterized by recurrent bacterial infections observed in patients with X-linked hyper-IgM syndrome who possess a defect in the gene encoding CD40L [48]. Platelet CD40L can also interact with neutrophils, and this interaction may be particularly relevant in the genesis of transfusion-related acute lung injury (TRALI). In this context, platelet-derived soluble CD40L may accumulate during platelet storage, which has the capacity to activate primed neutrophils in the lungs of predisposed patients, resulting in endothelial damage and possibly TRALI, as discussed below [49]. In addition to the aforementioned interactions with hematopoietic cells, CD40L can also modulate inflammatory responses though its interaction with endothelial cells, whereby CD40L binding to its cognate receptor CD40 on endothelial cells results in up-regulation of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and P-selectin as well as the release of interleukin (IL)-6 and tissue factor [9]. In addition, CD40L inhibits endothelial nitric oxide (NO) production by destabilizing NO synthase mRNA [42,50]; thus, CD40L binding to endothelial cells results in a proinflammatory phenotype [42]. Therefore, through its interactions with multiple cell types, CD40L is at the forefront of modulating immune and inflammatory responses.

Platelet-Endothelial Adhesive Interactions in Inflammation

It is increasingly recognized that inflammatory stimuli facilitate sustained endothelial-platelet adhesion by perturbing the antiadhesive properties of quiescent endothelium and inducing increased surface expression of endothelial adhesion molecules [51]. The adhesion of platelets to "inflamed endothelium" involves a similar coordinated multistep process as occurs in hemostasis and thrombosis, including platelet tethering, surface translocation, and firm adhesion (Fig 1) [52]. The expression of the endothelial selectins, P- and E-selectin, the membrane expression of VWF and ICAM-1 bound fibrinogen are all important in mediating platelet adhesion to the inflamed endothelium. The counter-receptors for P-selectin include GPIbα on platelets and P-selectin glycoprotein-1 (PSGL-1) on leukocytes [53,54], which support tethering and rolling, whereas it is thought that stable platelet adhesion is contingent upon platelet $\alpha_{IIb}\beta_3$ binding to fibrinogen.

Platelet-Derived Chemical Signals in Endothelial Activation

Platelets activated through their interactions with inflamed endothelium release a vast array of inflammatory and mitogenic mediators (see Table) into the local microenvironment. These platelet-derived

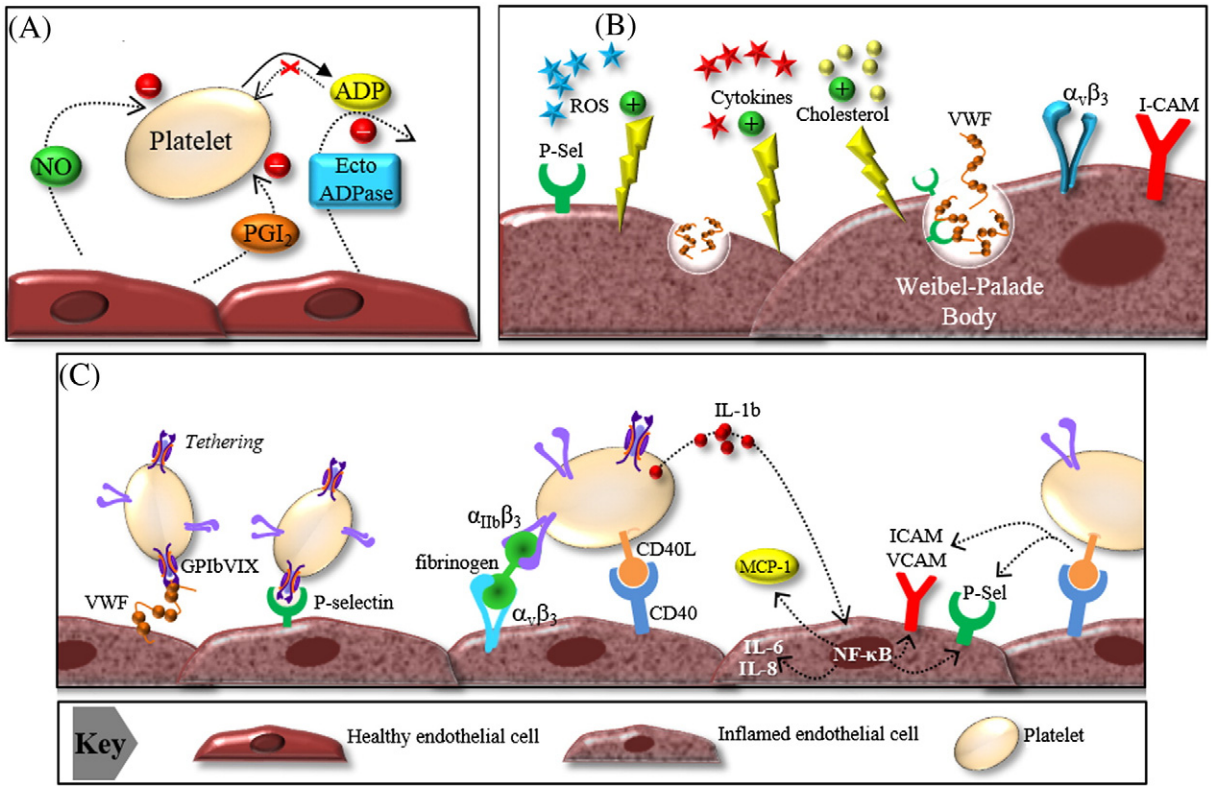

Fig 1. Platelet endothelial interactions in health and in inflammation. A, The healthy endothelium: the antiadhesive phenotype of endothelial cells is maintained through 3 intrinsic pathways; ecto-ADPase/CD39/NTPDase, which metabolizes ADP, and the PGI_2 and NO pathways, which inhibit platelet activation through stimulation of cAMP and cGMP production, respectively. B, Inflamed endothelium–endothelial activation: in infective or highly oxidative states as well as in the presence of a hyperlipidaemic milieu the endothelium becomes inflamed as a result of modified lipoprotein particles, cytokines, and reactive oxygen species accumulating in the intima. This leads to the expression of adhesive ligands (VWF and P- and E-selectins) on the endothelium. C, Platelet adhesion to the inflamed endothelium and platelet endothelial crosstalk: expression of VWF and P-selectin on the endothelial surface supports platelet tethering and rolling. Subsequently, stable platelet adhesion occurs through fibrinogen bridging of $\alpha_v\beta_3$ or ICAM-1 on endothelial cells with $\alpha_{IIb}\beta_3$ on the platelet surface. Adherent platelets secrete numerous bioactive substances which alter the chemotactic and adhesive properties of the endothelial cells. Platelet-derived IL-1β induces endothelial secretion of IL-6 and IL-8 as well as surface expression of ICAM-1, $\alpha_v\beta_3$, and MCP-1. Platelet CD40L binds CD40 on endothelial cells resulting in up-regulation of adhesive molecules (ICAM-1, VCAM-1, E-selectin, and P-selectin), cytokine, and tissue factor release and reduction in NO synthesis. Figure adapted from Kaplan and Jackson [85] with permission.

bioactive substances alter the chemotactic and adhesive properties of the endothelial cells, enabling monocytes and other leukocytes to adhere and transmigrate through the endothelium to sites of inflammation [55,56]. In vivo models of atherogenesis (see below), a prototypic disease of thromboinflammatory dysregulation, suggest that these interactions between endothelial cells and activated platelets are likely to be critical in the initiation of atherosclerosis [57,58].

Platelet-Leukocyte Crosstalk in Inflammation

There is increasing evidence that activated platelets adherent to the inflamed endothelium may enhance leukocyte recruitment, activation, and transmigration, thereby enhancing the inflammatory processes (Fig 2), which are central to the genesis of clinically relevant thromboinflammatory diseases such as disseminated intravascular coagulation, transplant rejection, and ischemia-reperfusion injury. Leukocyte recruitment to endothelial-bound platelets is a coordinated multistep process involving selectin-mediated tethering and rolling, followed by β_2 integrin–mediated stable adhesion. Platelet P-selectin has a central role in mediating the initial capture and rolling of leukocytes through interactions with its key receptor, PSGL-1 [53,59]. Ligation of PSGL-1 induces signaling events culminating in the activation of the leukocyte β_2 integrins including macrophage antigen-1 (Mac-1; $\alpha_M\beta_2$) and lymphocyte function–associated antigen-1 (LFA-1; $\alpha_L\beta 2$), which are required to mediate stable leukocyte adhesion. Macrophage antigen-1 is the principal β_2 integrin that facilitates firm adhesion onto the surface of

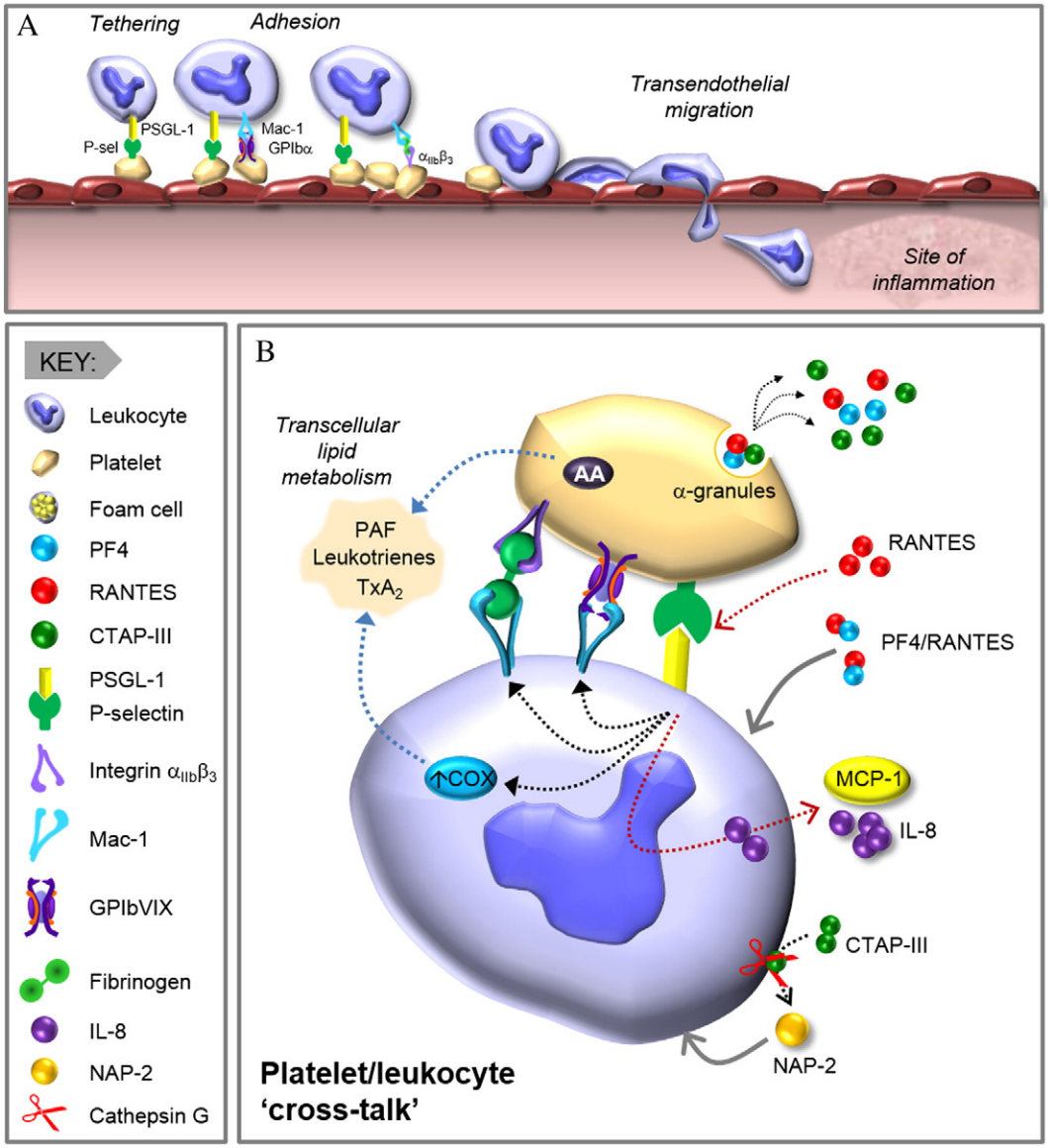

Fig 2. Platelet-leukocyte interactions in inflammation. A, Platelet-mediated leukocyte recruitment and adhesion: leukocyte recruitment to endothelial-bound platelets occurs in a multistep coordinated process. Initial tethering of leukocytes is mediated by the interaction of P-selectin expressed on the platelet surface with its cognate receptor leukocyte PSGL-1. Ligation of PSGL-1 promotes activation of leukocyte β_2 integrins (MAC-1 and LFA-1), necessary for stable leukocyte adhesion. Note that Mac-1 can engage several platelet ligands including GPIbα, ICAM-2, and JAM-3, as well as fibrinogen bound to $\alpha_{IIb}\beta_3$. Leukocytes subsequently undergo transendothelial migration to sites of infection and inflammation. B, Platelet leukocyte crosstalk: bioactive mediators derived from α-granules of activated platelets, including PF4, synergizes with RANTES to enhance leukocyte adhesion and monocyte differentiation. RANTES acting in concert with P-selectin results in MCP-1 and IL-8 secretion by monocytes. CTAP-III is another chemokine released from the platelet α-granules, which is subsequently converted into active NAP-2 by the neutrophil membrane associated serine-protease cathepsin G. NAP-2 enhances leukocyte adhesion and neutrophil transendothelial migration and, within the developing thrombus, provides a chemokine gradient guiding leukocytes toward the site of vascular injury. Up-regulation of COX-2 in monocytes via pathways involving IL-lβ and P-selectin results in increased production of proinflammatory mediators including PAF, leukotrienes, and TXA_2 via transcellular eicosanoid metabolism. Figure adapted from Kaplan and Jackson [85] with permission.

platelets via its binding to multiple ligands including GPIbα [60], ICAM-2 [61], and junctional adhesion molecule-3 (JAM-3) [62] as well as through fibrinogen bound to $\alpha_{IIb}\beta_3$ [56,63].

Platelet activation with consequent degranulation results in liberation of numerous chemokines stored in platelet α-granules (see Table). These chemokines can either be released into the circulation or displayed on the platelet surface where they exert numerous biological activities instrumental in mediating inflammatory and immune responses (Fig 2) [64]. Classically, chemokines are divided into 4 families (CXC, CC, CX3C, and XC) based on the arrangement of the N-terminal cysteine residues [65]. The CC and CXC bind exclusively to CC and CXC chemokine receptors, respectively; however, within families there is considerable promiscuity relating to binding of chemokines to chemokine receptors [64]. Platelet granules contain both CXC (PF4, growth-regulating oncogene-α [GRO-α], epithelial neutrophil-activating protein-78 [ENA-78], and CXCL7) and CC chemokines (RANTES, macrophage inflammatory protein-1α [MIP-1α], and monocyte chemotactic protein [MCP]-3), with platelets being the most abundant and readily available source of CXC chemokines [66]. In addition to releasing prestored chemokines and cytokines, platelets also participate in transcellular metabolism of eicosanoids with endothelial cells and leukocytes, leading to the local synthesis of proinflammatory lipids including platelet-activating factor (PAF), leukotrienes, and TXA_2 [67].

In recent studies, we have identified a major role for the platelet chemokine neutrophil-activating peptide 2 (NAP-2; CXCL7) in regulating leukocyte polarization and motility [68]. CXCL7 is the most abundant platelet chemokine that appears in serum at micromolar concentration; this is 2-fold greater than PF4, the next most abundant, and several orders of magnitude greater than most other platelet chemokines [69]. CXCL7 has several molecular variants that are derived by sequential N-terminal proteolytic events [70]. Interestingly, NAP-2 is only generated in the presence of leukocytes through proteolytic cleavage by the neutrophil membrane-associated serine-protease cathepsin G [71] and mast cell chymase [72]. Furthermore, NAP-2 represents the only CXCL-7 derivative that exhibits typical chemokine properties [69]. Although NAP-2 has been demonstrated to induce neutrophil and monocyte adhesion in vitro as well as neutrophil transendothelial migration [64,73] and elastase release [74], our studies provide the first in vivo evidence that graded platelet activation within a developing thrombus results in the generation of a NAP-2 chemotactic gradient within the thrombus body, guiding leukocytes to sites of vascular injury.

Additional CXC chemokines expressed in platelets include CXCL4 (PF4), CXCL1 (GRO-α), CXCL5 (ENA-78), and CXCL8 (IL-8). PF4 is the second most abundant platelet chemokine; however, it does not exert classical chemokine functions at submicromolar concentrations [64,75] on its own, but rather acts together with other platelet-derived chemokines such as RANTES to modulate leukocyte responses. CXCL1 is not present exclusively in platelets, but it is also expressed by neutrophils, monocytes, and endothelial cells [64,76]. CXCL1 is able to promote both neutrophil recruitment [77] and arrest of monocytes under flow conditions [78]. CXCL5 has significant structural homology with NAP-2 [79] and shares its ability to induce neutrophil chemotaxis [80] with a similar potency to IL-8 [81]. In addition to being a chemoattractant for neutrophils, IL-8 is also able to stimulate neutrophil degranulation and enhance expression of Mac-1 [82]. Interleukin-8 is also a chemoattractant for T cells and basophils and also induces histamine release [83]. It, however, is not produced exclusively by platelets [84].

Platelets as Mediators of Inflammatory Disease

Atherosclerosis

The concept that platelets may have proinflammatory functions emerged from extensive experimental work highlighting their role in the pathogenesis of atherosclerosis. Consequently, the role of platelets in atherogenesis has been the focus of recent comprehensive reviews [52,55,85,86] and therefore will only be discussed briefly. The adhesion of platelets to sites of injured and atherosclerosis prone endothelium may conspire to accelerate the progression of atherosclerotic lesions. Adherent platelets release a number of chemokines (CCL5), cytokines (IL-1β),and other biologic response modulators (CD40L) that act to enhance the recruitment of proatherogeneic monocytes. In addition, adherent platelets may release growth factors, such as platelet-derived growth factor (PDGF), which may enhance angiogensis and smooth muscle proliferation in atherosclerotic plaques.

In addition to enhancing atherogenesis, platelets may also contribute to atherothrombosis by augmenting plaque instability via CD40L binding to smooth muscle cells, endothelial cells, and monocytes, which induce expression of matrix metalloproteinases [50]. Matrix metalloproteinase possesses collagenase activity and thus may cause degradation of the extracellular matrix collagen culminating in the thinning of the fibrous cap and consequent plaque instability. Consistent with this hypothesis, enhanced collagen content has been observed in the extracellular matrix of atherosclerotic lesion in murine models in which CD40L signaling has been interrupted [87,88]. Furthermore, though controversial, CD40L may be important in forming the necrotic core of the atheromatous plaque through induction of proapoptotic pathways [50]. Therefore, CD40L contingent mechanisms may enhance plaque, instability making them more susceptible to rupture.

Transfusion-Related Acute Lung Injury

Transfusion-related acute lung injury is defined as the new onset of acute lung injury within 6 hours of transfusion. Clinically, TRALI may manifest as hypoxia, fever, chills, tachycardia, and bilateral noncardiogenic pulmonary odema and is the leading cause of transfusion-associated mortality [89,90]. The central role of neutrophils in mediating TRALI has long been appreciated. Indeed, this has led to a "2-hit" model of neutrophil-induced acute lung injury, such that the first hit represents the susceptible host with underlying pulmonary pathology, with the second hit being a transfusion-related insult [89,90]. The "first hit" results in endothelial activation which facilitates neutrophil adhesion via the up-regulation of endothelial adhesion molecules and chemokine synthesis. The "second hit" then results in the activation of adherent neutrophils which leads to degranulation and release of mediators that cause endothelial damage and increased vascular permeability. In the context of TRALI, the second hit that causes neutrophil activation can include antineutrophil antibodies or bioactive lipids such as lysophosphatidylcholine. The source of these mediators that may induce the second hit is thought to either be donor derived (antineutrophil antibodies) or result from the breakdown of biological membranes, which may occur during the storage process of blood products. More recently, experimental data have demonstrated that soluble CD40L (also known as CD154) may be released by stored platelet concentrates and promote neutrophil activation via activation of neutrophil CD40 receptors [49]. Platelets have also been demonstrated to be directly involved in the pathogenesis of TRALI in murine models by way of their ability to bind to neutrophils and instigate NET formation [91]. The importance of platelets in this process was highlighted by the ability of platelet inhibition to protect from the development of TRALI [91]; however, the precise mechanisms leading to NET formation in TRALI are yet to be established

Immune Thrombocytopenic Purpura (ITP)

Immune thrombocytopenic purpura (ITP) is perhaps the classical immune condition that one associates with platelets. It is associated with immune dysregulation and involves antigen-presenting cells, B cells, and T cells acting in concert to perpetuate an autoimmune response that hastens the destruction of platelets and/or perturbs megakaryopoiesis [92,93]. It has long been observed that ITP is associated with infections, and more recent data suggest that platelet TLR4

expression may play a central role in this process [32,94]. As discussed above, platelets express all of the TLRs and TLR4 is thought to be responsible for mediating thrombocytopenia and TNF-α production in response to LPS. In the context of ITP, recent evidence has demonstrated that gram-negative LPS can bind to platelet TLR4 and synergize with antiplatelet antibodies, thus enhancing phagocytosis in vitro and thrombocytopenia in vivo [32]. This may explain why some patients with ITP experience lower platelet counts with intercurrent infection, which may abate with antibacterial treatment.

Transfusion-Related Immunomodulation

The clinical observation that blood transfusion may be associated with an increase in infection risk or tumor growth or improved outcomes after renal allograft transplantation has given rise to the concept of transfusion-related immunomodulation [95]. Although there may be multiple ways the immune system could be modulated by blood transfusion, there are now data supporting a major role for platelets in this process. Although platelets are devoid of MHC class II molecules, they express a significant amount of MHC class 1 [96]. Surprisingly, there appears to be 2 distinct sources of platelet MHC class 1 molecules. Platelets contain both surface-derived, denatured class 1 MHC molecules that are adsorbed by platelets from the plasma. Furthermore, it has recently been demonstrated that platelets contain all the necessary machinery (such as MHC mRNA and proteasomes) for class 1 MHC expression and antigen presentation [97]. Interestingly, functional consequences of MHC class 1 molecule expression appear somewhat divergent. In the context of transfusion medicine, denatured MHC class 1 molecules can be shed from the surface of stored platelets; however, these shed allogeneic MHC class 1 molecules do not appear able to generate a cytotoxic T cell response and have been demonstrated to improve the survival of allogeneic skin grafts in mouse models [98]. On the other hand, the platelets' ability to process antigenic material and express it in a MHC class 1 dependent manner has recently been shown in a mouse model of cerebral malaria to play a central role in both the acquired immune response and in enhancing cytotoxic T-cell activation [97].

Sepsis

Thrombocytopenia has long been considered a manifestation of sepsis and is associated with increased mortality [9]. Recent experimental evidence now suggests that platelets play an active role in the pathogenesis of sepsis and multiorgan dysfunction. Elevated circulating levels of platelet-leukocyte aggregates have previously been reported in a broad range of inflammatory conditions including sepsis [15,67,99]. Platelet-neutrophil aggregates comprise activated platelets bound to leukocytes. These aggregates are supported by interactions between platelet P-selectin and GPIbα and their respective leukocyte counter receptors, PSGL-1 and Mac-1 [100]. In addition, plasma from septic but not healthy patients can induce platelet-neutrophil interactions in vitro [36]. These interactions are likely highly contingent upon stimulation of the platelet TLR4 receptor given the observation that the use of the TLR4 antagonist eritoran resulted in a 50% reduction in platelet-neutrophil interactions [36].

The functional consequences of platelet-leukocyte interactions are elucidated by the recent observation that LPS-stimulated platelets bind to neutrophils and stimulate the formation of NETs. Neutrophil extracellular trap formation represents a mechanism used by neutrophils to entrap and possibly kill pathogens [101]. This neutrophil killing strategy involves the active extrusion of nuclear and cytoplasmic granular constituents that combine to form NETs [101]. Neutrophil extracellular traps are composed of chromatin (DNA and histones) fibers that are coated with proteins from azurophilic granules (neutrophil elastase, cathepsin G, and myeloperoxidase) as well as proteins from specific and tertiary granules, such as lactoferrin and gelatinase [101]. Neutrophil extracellular traps are highly efficient at trapping and killing gram-negative and gram-positive bacteria as well as mycobacteria, yeast, fungi, and protozoan [102]. However, exaggerated NETs release can have deleterious consequences, resulting in inflammatory and autoimmune diseases [102], as well as induction of pathological thrombosis [103–105]. Induction of NET release from leukocytes by LPS-stimulated platelets is strikingly efficient and rapid, occurring within minutes of exposure [36]. This contrasts with other NETs inducing stimuli such as sepsis, cytokines, and reactive oxygen species [102,106,107], which promote NET formation by neutrophils typically after 2 to 4 hours [108]. Platelets promote NET release through the LPS receptor TLR4, suggesting that platelet TLR4 may represent "a threshold switch" for bacterial trapping by NETs in severe sepsis [36].

In addition to inducing NET formation, activated platelets from septic patients have enhanced microparticle formation [109,110]. Microparticles are small (0.1-1 μm) fragments of membrane shed from different cell types including platelets, leukocytes, and endothelial cells [111]. Platelet microparticles constitute approximately 70% to 90% of circulating microparticles [112]. Microparticles shed from activated platelets can express adhesion molecules such P-selectin and $\alpha_{IIb}\beta_3$ and therefore can adhere to and activate endothelial cells and leukocytes [110]. Importantly, microparticles may contain potent proinflammatory molecules such as IL-1β and CCL5, which, when shed from activated platelets, may communicate proinflammatory signals to extravascular tissue [112,113]. Platelet-derived microparticles also contain small non–protein-coding (21-23 nucleotides) microRNAs which are able to modulate cellular function and protein expression via degradation of mRNA or repression of mRNA translation [114,115]. Platelet microparticles are able to transfer microRNAs to cells types including endothelial cells and leukocytes [116] and thus through this pathway may modulate cellular functions involved in immunity and inflammation as well as in hematopoiesis, developmental biology, angiogenesis, and malignancy [114,115].

Cancer

A relationship between thrombocytosis and malignant tumors was first observed more than 100 years ago by Reiss et al in 1872 [117]. More recently, clinical data have demonstrated that an elevated platelet count is associated with more advanced cancer in addition to being an adverse prognostic marker [118–120]. There is now a growing body of evidence demonstrating a key role for platelets in the pathogenesis of tumor progression and metastasis. Tumor cells can aggregate platelets in vitro (or tumor cell–induced platelet aggregation) [121,122]. This property of tumor cells correlates with their ability to metastasise in vivo. Furthermore, platelet depletion or inhibition of platelet function has been shown to inhibit tumor metastases in murine models [121,123]. It is proposed that the ability of tumor cells to aggregate platelets gives rise to several survival advantages in vivo. Platelet adhesion to tumor cells may form a "shield" around tumor cells entering the circulation and therefore confer protection from immune clearance [124,125]. In addition, interaction of metastasising tumor cells in the circulation with endothelial cells is enhanced by the presence of bound platelets [126]. Furthermore, platelets may release growth factors contained in their granules such as PDGF, vascular endothelial growth factor, and angiopoietin 1, which can facilitate tumor growth [127,128]. Based on experimental models where deficiency or inhibition of the platelet $\alpha_{IIb}\beta_3$, GPVI, or P selectin resulted in a decreased frequency of tumor metastasis, these are thought to be the key platelet receptors facilitating platelet-tumor interactions [127,128]. In addition to playing a role in platelet-tumor interactions, $\alpha_{IIb}\beta_3$ activation is also believed to be essential for the tumor growth enhancing of platelets, as activation of this integrin appears necessary for the release of proangiogenic factors from the platelet which support tumorigenesis [129].

Rheumatoid Arthritis

Electron microscopic analysis of the synovium from patients with rheumatoid arthritis (RA) more than 30 years ago demonstrated platelet thrombi in synovial vessels [130]. Furthermore, these studies identified platelets in the vicinity of gap junctions of synovial vessels. More recently, considerable evidence has accumulated highlighting enhanced platelet activation in patients with RA. In samples from patients with RA, it has been shown that platelets express higher levels of P-selectin, display enhanced responsiveness to soluble agonists in vitro, and demonstrate elevated levels of circulating platelet microparticles and platelet-leukocyte aggregates [131–134]. Furthermore, platelet aggregates and platelet-adherent leukocytes have been observed in the synovial fluid of patients with RA [135,136]. These observations suggest that platelets may not merely be passive bystanders in RA pathogenesis but rather may be key players in its pathogenesis.

Fundamental new insights into the pathogenic role of platelets in RA were provided in a pivotal study by Boilard et al [137]. Surprisingly, examination of synovial fluid from patients with RA demonstrated that the synovial fluid in RA is enriched in platelet microparticles rather than whole platelets [137]. Importantly, the concentrations of platelet-derived microparticles are much higher in the synovial fluid than in the blood, suggesting that they form locally in the joint vasculature. The key stimulus for platelet activation and platelet microparticle formation in RA appears to be the interaction of platelet GPVI with collagen and laminin [137]. Activation of platelet GPVI induces the release of platelet-derived microparticles containing IL-1 which can induce fibroblast-like synoviocytes to release the proinflammatory cytokines IL-6 and IL-8 [137]. The central role for the GPVI-induced platelet activation axis in promoting inflammatory arthritis is evidenced by murine models where deletion of platelet GPVI or platelet depletion resulted in a marked reduction in the level of arthritis activity [137]. Although the precise mechanism by which microparticles enter the synovial space is yet to be fully elucidated, it is conceivable that neutrophils with adherent platelet-derived microparticles may act to shuttle microparticles into the synovial fluid. Alternatively, owing to their small size, microparticles may enter the synovial space by diffusing across dilated endothelial gap junctions—a process that itself is exaggerated by serotonin released by platelets in arthritic joints [138].

Summary and Perspectives

For more than a century, platelet research has focused primarily on defining the hemostatic and thrombotic roles of platelets. However, studies into the nonhemostatic properties of platelets are in relative terms, in their infancy. As a corollary, just as expanding scientific knowledge on the prothrombotic role of platelets has enabled the development of a broad range of antiplatelet drugs that are widely used in cardiovascular disease, it is envisaged that enhancing our understanding of the proinflammatory and immune modulating roles of platelets will facilitate the development of interventions to therapeutically target these nonhemostatic roles of platelets.

Conflict of Interest Statement

The authors declare no relevant conflict of interest.

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