Published in final edited form as:

Circ Res. 2018 January 19; 122(2): 337–351. doi:10.1161/CIRCRESAHA.117.310795.

Circulating Platelets as Mediators of Immunity, Inflammation and Thrombosis

Milka Koupenova[1,*], **Lauren Clancy**[1], **Heather A. Corkrey**[1], and **Jane E. Freedman**[1]

[1]University of Massachusetts Medical School, Department of Medicine, Division of Cardiovascular Medicine, Worcester, MA 01605, USA

Abstract

Platelets, non-nucleated blood components first described over 130 years ago, are recognized as the primary cell regulating hemostasis and thrombosis. The vascular importance of platelets has been attributed to their essential role in thrombosis, mediating myocardial infarction, stroke, and venous thromboembolism (VTE). Increasing knowledge regarding the platelets' role in the vasculature has led to many advances in understanding not only how platelets interact with the vessel wall but how they convey changes in the environment to other circulating cells. In addition to their well-described hemostatic function, platelets are active participants in the immune response to microbial organisms and foreign substances. Although incompletely understood, the immune role of platelets is a delicate balance between its pathogenic response and its regulation of thrombotic and hemostatic functions. Platelets mediate complex vascular homeostasis via specific receptors and/or granule release, RNA transfer, and mitochondrial secretion that subsequently regulates hemostasis and thrombosis, infection, and innate and adaptive immunity.

Keywords

platelets; communication; immunity; infection; thrombosis

Introduction

After erythrocytes, platelets are the most prevalent blood component, and they vary in size from 2–5 μm[1]. Once released by their megakaryocytic precursor, platelets enter the bloodstream and circulate for 7–10 days[2, 3]. Platelets are complex cells that contain three distinct types of granules: α-granules, dense or δ-granules, and lysosomes[4, 5]. Platelet α-granules contain various proteins, chemokines, cytokines and growth factors that are assembled in platelets by megakaryocytes and are necessary for normal platelet functionality. Platelet δ-granules contain small molecules such as ADP, serotonin, polyphosphates, glutamate, histamine and calcium that are necessary for hemostasis[4, 6]. Platelet lysosomes contain enzymes such as glycohydrolases and enzymes that degrade

*Corresponding Author. **Milka Koupenova**, University of Massachusetts Medical School, Albert Sherman Center, 368 Plantation St, AS7-1014, Worcester, MA 01605, Tel: (508) 856-6937, Fax: (508) 856-6933, milka.koupenova@umassmed.edu.

Disclosures

None

glycoproteins, glycolipids and glycosaminoglycans[4, 7]. Platelets do not have a nucleus but contain prepackaged proteins and various forms of RNA from their precursor cells. Found throughout the vasculature, platelets respond to signals from the endothelium, circulating cells, or other blood components. In this review, we will focus on the complex roles of platelets in thrombosis and immunity facilitated by their various interactions with the endothelium and circulating immune cells.

Established mechanisms for hemostasis and thrombosis

It is well established that in primary hemostasis, platelets adhere to the damaged vessel wall at the site of injury[2, 3, 8]. This process occurs through multiple signaling cascades and is highly dependent on glycoproteins (GPs) expressed on the platelet surface[2, 3, 8]. The inhibitory function of the intact endothelium (specifically, the production of prostacyclin and nitric oxide as well as expression of CD39) is decreased upon vessel damage, and extracellular matrix proteins such as collagen are exposed to the circulation[2, 3].

Platelet adhesion, or initial binding of platelets to the damaged site, differs depending on the level of shear stress in the circulation. At high shear stress, circulating plasma-derived von Willebrand factor (vWF) rapidly unfolds and deposits on the exposed subendothelial collagen at the injury site. The unfolding of vWF exposes multiple binding sites for GP1b (CD42), a member of the GP1b-V-IX receptor complex[2, 3, 8]. This process decreases platelet velocity allowing for low shear stress and GPVI-collagen interactions[2, 3, 8]. Under low shear stress, platelets adhere predominantly to collagen through their GP1a/2a integrins. This phase of adhesion leads to platelet activation as well as a number of distinct platelet physiological and cytoskeletal changes, including a shift from a resting discoid shape to an activated state with numerous pseudopodia (Figure 1)[2, 3, 8].

The initial adhesion and activation of platelets is followed by recruitment of additional platelets from the circulation and formation of three-dimensional aggregates through a number of molecular interactions. The recruitment of other platelets, their activation, and platelet aggregation is mediated by platelet generation of thromboxane A2 (TxA2) and release of ADP from their δ-granules. Platelet-platelet interactions during thrombi formation are further mediated and stabilized by the platelet fibrinogen receptor GP2b/3a[2, 3, 8].

Depending on the level of the injury, blood can leak into the tissue and expose platelets to tissue factor expressed by cells that comprise the vessel. The presence of tissue factor leads to thrombin generation which mediates firm platelet aggregation. Once formed, the primary hemostatic plug retracts and becomes firmly anchored at the site of injury, preventing re-bleeding. Clot stabilization is described as secondary hemostasis and is characterized by consolidation of the platelet mass through actin/myosin-mediated platelet retraction. At this stage, thrombin, in addition to activating platelets, converts fibrinogen to fibrin leading to the formation of a network of fibrin fibers. In certain settings, normal hemostasis is overwhelmed by pathological factors leading to uncontrolled clot formation and potential vessel occlusion. As previously discussed, uncontrolled clot formation is defined as thrombosis and, in the artery, is primarily mediated by platelets. Arterial thrombosis is a

central cause of myocardial infarction (MI) and stroke (embolic or *in situ*), while venous clot formation may lead to deep vein thrombosis and/or venous thromboembolism (VTE).

In response to blood borne pathogens and consequent tissue damage, platelets are also involved in a coordinated intravascular coagulation response recently termed "immunothrombosis". During this process, platelets and immune cells form a physical barrier of confinement preventing dissemination of pathogens and potentially leading to activation of the innate and adaptive branches of the immune system[6, 9]. Interestingly, platelets mediate the crosstalk between the hemostatic and the immune system utilizing similar pathways (Table 1). Consistently, various viral or bacterial infections have been found to contribute to the risk of thrombosis that manifests as arterial thrombosis or VTE and may contribute to atherosclerosis[10–12]. Thus, although immunothrombosis may be an efficient way of assisting the immune system, it may significantly contribute to the overall risk of cardiovascular disease.

Contemporary mechanisms for hemostasis and thrombosis

Recent *in vivo* studies have provided a more complex view of hemostasis and thrombosis. This new model suggests that the hemostatic plug is characterized by a stringent architecture, with a distinct core and outer shell[13, 14]. Fibrin deposition is distinctly localized at the base of the core in the extravascular space before complete hemostasis is achieved. A platelet activation gradient is also established, with the inner core of the plug composed of tightly packed, activated platelets which are degranulated and P-selectin positive, while the outer shell is composed of less activated loosely packed platelets that do not express P-selectin[13, 14]. The outer shell, however, is stable and permeable to plasma solutes. Consistent with the activation-gradient of platelet distribution, there is a distinct distribution of platelet agonists throughout the thrombus. The core of the plug contains a high concentration of thrombin and, as the plug becomes more porous, a gradient of ADP and TxA2 develops[13, 14].

The porous outer shell of the thrombus may allow for recruitment of leukocytes necessary for injury repair or pathogen elimination. At the site of injury in veins (low shear stress), in addition to platelet activation and thrombus formation, thrombin plays a central role in leukocyte recruitment to the hemostatic plug. This process is mediated predominantly by activated platelets and not endothelial cells[15]. Activation/cleavage of the platelet thrombin receptor PAR4 promotes leukocyte recruitment and migration[15] and platelet P-selectin enables leukocyte interaction with the thrombus[15, 16]. Thrombin also mediates fibrin generation which limits leukocyte migration in the clot by forming a physical barrier[15]. Finally, platelet GP1b can bind thrombin depleting its effect and limiting leukocyte trafficking into the thrombus[15].

Under lower shear stress conditions either in veins or in arteries (ischemia reperfusion), platelet adhesive interactions can also lead to formation of a CXCL7 chemotactic gradient within the thrombus body. This gradient guides intravascular migration of leukocytes through the thrombi to the sites of injury via their CXCR1/2 receptors[16]. This suggests that platelets, by mediating hemostasis, can also facilitate leukocyte recruitment to the clot

enabling close contact of the immune system with potential pathogens that may have penetrated the skin barrier. Further studies are necessary to elucidate how platelet distribution changes *in vivo* in pathogen-associated venous thrombi.

Platelets and endothelial cells

The interaction between platelets and endothelial cells has been previously and extensively reviewed[17, 18]. Platelet rolling over intact endothelium is observed predominantly in veins and increases with endothelial activation in response to inflammatory stimulus or infection. Under low shear stress in veins, platelet-endothelial interactions are mediated by platelet-GP1bα and endothelial-vWF[18]. Under higher shear stress in small venules, endothelial P-selectin and platelet PSGL1 or GP1bα mediate platelet rolling[18]. In the absence of additional signals, platelets disengage and return to the circulation. When the endothelium is under stress, firm adhesion of platelets will occur through endothelial ICAM1 (or through endothelial αVβ3) and platelet GP2b/3a in a fibrinogen-dependent manner. Endothelial stress is manifested during inflammation or infection by an increase in endothelial surface expression of P-selectin, E-selectin, VCAM and ICAM-1[19]. Endothelial cells also regulate platelet activation and thrombus size by secreting thrombo-regulators such as nitric oxide (NO) and prostacyclins, and removing ADP/ATP through CD39-ectonucleotidase. NO can also be secreted by platelets and can inhibit additional platelet recruitment, reduce P-selectin expression and promote disaggregation[20].

In addition to integrin/selectin surface expression, platelets can also affect the endothelium by increasing their surface expression of CD154 (CD40L) or by secreting cytokines such as IL-1β. Seconds after thrombin stimulation of human platelets *in vitro*, CD154 surface expression occurs. Platelet CD154, in turn, can lead to expression of endothelial E-selectin, VCAM1 and ICAM1, as well as secretion of MCP1 and IL-8 from endothelial cells[21]. In addition to surface protein expression, platelets can synthesize proteins from stored RNA templates[22, 23]. One of these proteins, the cytokine IL-1β, is synthesized in response to inflammatory stimuli[22] (Figure 2). Released IL-1β can increase endothelial permeability, as well as recruitment and attachment of leukocytes to the endothelium[24, 25]. This leads to platelet alteration of the inflammatory cell transmigration to damaged or infected tissue. Interestingly, IL-1β increases the binding potential of platelets to collagen and fibrinogen and, in the presence of collagen, promotes aggregation[26].

Receptors in platelet-immune cell interactions

To understand the role of platelets in thrombosis in diverse clinical settings, their role in immunity should be addressed. Under certain infectious conditions or inflammatory stimuli, platelets directly interact with circulating leukocytes by changing surface expression of P-selectin or CD40[5]. Platelet P-selectin and CD40 are recognized by leukocyte PSGL1 and CD154, respectively, leading to formation of platelet-leukocyte aggregates. Platelets form these aggregates predominantly with circulating monocytes or neutrophils, thereby contributing to the innate immune response[5]. Platelets also contribute to adaptive immunity by interacting with and activating dendritic cells (DCs) through the CD40-CD154 axis. Platelet-mediated activation of DCs causes the DCs to present antigen to T-cells[5]. In

addition, platelet activation can also lead to the release of δ-granule content and secretion of molecules such as serotonin and RANTES (CCL5) that are also known to mediate T-cell activation and differentiation[27–29]. Notably, platelets contain the mRNA transcripts for all Toll-like receptors (TLR1-TLR10)[4, 30, 31]. TLRs are pathogen-associated molecular pattern recognition receptors that are key regulators in the initiation of the innate immune response to foreign organisms.

Platelets express functional TLR4[32–34], TLR2[35–37] and TLR9[38, 39], and these TLRs can initiate thrombotic responses of varying intensities (Table 2). Platelet-TLR4 and -TLR2 can also mediate interactions with neutrophils[33, 36]. Additionally, platelets express functional TLR7[40] and TLR3[41, 42]; however, these TLRs do not induce prothrombotic responses (aggregation mediated by thrombin[40]), but instead alter platelet-leukocyte interactions. Stimulation of TLR7 leads to the platelet surface expression of P-selectin and CD154, suggesting select α-granule release[40]. Stimulation of TLR3 does not lead to P-selectin or CD154 surface expression but, at high agonist concentration (possibly equivalent to late stages of infection), potentiates arachidonic acid-, ADP- or collagen-mediated aggregation[41, 42]. Interestingly, analysis of platelets from 1864 people shows that women have significantly higher expression of all TLRs as compared to men[30]. TLR expression in platelets from women associated with an increase of plasma P-selectin while, in men, TLR expression associated with an increase in IL-6, TNFRII and soluble ICAM-1[30].

Importantly, platelet immune activation differs from hemostatic, thrombin-mediated platelet responses. Scanning electron micrographs of human platelets alone and in the presence of leukocytes, illustrate a distinct morphological shape change dependent on their function (Figure 1)[40]. Platelet hemostatic function demonstrated by stimulation with thrombin causes complete disappearance of the platelet's discoid shape and conversion into long intertwined "spaghetti-like" pseudopodia (Figure 1). Platelet immune function demonstrated by stimulation with TLR7 agonist or TLR7-activating virus leads to smaller platelet groups in which single platelets can be identified with fewer pseudopodia connecting to other platelets or to leukocytes (Figure 1). This suggests that platelets undergo distinct forms of activation depending on the stimulatory signal and their functional role. Different levels of activation appear necessary for the proper formation of either the hemostatic plug or recruitment of leukocytes (see "Contemporary mechanisms for hemostasis and thrombosis").

Platelets and neutrophils

The platelet interaction with neutrophils is central to initiating the immune response (Figure 2). In humans, neutrophils are the most abundant blood leukocyte and, together with monocytes, are the major initiators of the innate immune response. Platelets form heterotypic aggregates with neutrophils as a function of TLR7, TLR2, and TLR4 activation in the circulation[33, 36, 40]. Platelet-neutrophil interactions are mediated by the platelet P-selectin/neutrophil PSGL1 axis. Neutrophils can also phagocytose activated platelets *in vivo* with internalization mediated by phosphatidylserine platelet surface expression[43]. Interestingly, as a result of TLR7 stimulation, platelet components (as measured by CD41) are internalized by neutrophils[40]. It is currently unclear whether platelet microparticles[44] or the entire platelet is internalized as a function of TLR7-stimulation[40].

Platelet-neutrophil interactions are also important for neutrophil extracellular trap (NET) formation, a process termed netosis whereby pathogens are captured and neutralized[45–47]. *Staphylococcus aureus* alpha toxin for instance mediates secretion of β-defensin 1 from platelets which can lead to NET formation[48]. In the setting of lipopolysaccharide (LPS) activation of TLR4, platelets contribute to netosis by reducing the time required for NET formation[33] (Table 2). Although various TLRs are implicated in organ netosis, the role of platelet TLRs, with the exception of TLR4, has not been elucidated (Table 2).

Platelets and monocytes

Platelets can interact with monocytes as a consequence of inflammatory stimuli or infection (Figure 2). Platelet-monocyte aggregates may be a more sensitive method for assessing platelet activation as compared to P-selectin expression[49]. Circulating platelet-monocyte aggregates are elevated in patients with acute myocardial infarction[50, 51] and, in older patients, platelet-monocyte aggregates are associated with vascular thromboembolism[52]. Murine studies have shed light on the complex interaction between platelets and monocytes. During chronic inflammation, simultaneous activation of platelets and neutrophils leads to secretion of RANTES and human neutrophil peptide 1 (HNP1), respectively[53]. These proteins form heterodimers responsible for monocyte/macrophage adhesion and recruitment in a model of myocardial infarction[53]. Thrombin-activated human platelets also express the chemokine receptor CX3CR1 leading to the formation of platelet-monocyte complexes through the CX3CL1 ligand on monocytes[54].

Platelets and eosinophils

Platelets are described to affect eosinophil function as well. Eosinophils are granulocytes that compose about 2% of all leukocytes and it has been proposed that they may contribute to the innate immune response to helminth parasite (worms) infection. Pathogenically, eosinophil function has been attributed to the profound damage they cause in an allergic asthma. Direct interaction between platelets and eosinophils in the circulation during helminth infection *in vivo* has not been described. However, co-incubation experiments of platelets and eosinophils (for 48h) show that platelets prolong eosinophil life by secreting granulocyte-macrophage colony-stimulating factor (GM-CSF)[55]. This suggests that platelets may enable eosinophils in fighting helminth infection. However, by delaying eosinophil apoptosis platelets may also contribute to asthma-mediated tissue damage. A murine model of allergic inflammation has suggested that platelets through their P-selectin recruit eosinophils to the lungs[56] and consequently may propagate tissue damage. Allergen sensitized mice had an increase of platelet-eosinophil aggregates in the circulation and in the lung tissue and aggregate interactions were mediated through platelet P-selectin and eosinophil PSGL1[56].

Platelets, B-cells and T-cells

When interacting with lymphocytes, platelets are more selective and bind preferentially to larger, activated cells[57]. In the presence of LPS or ADP, aggregation between platelets and B-cells is almost unaffected. However, when platelets are co-incubated with B-cells for three

days *in vitro*, B-cells increase their production of IgG1, IgG2 and IgG3, suggesting that platelet content can contribute to B-cell function and alter adaptive immunity[58].

T-cell activation more robustly increases platelet aggregation via both T-cytolytic and T-helper cells[57] (Figure 2). This process is mediated by platelet GP2b/3a, CD154, and lymphocyte CD11b[57]. CD154 is expressed under various stimulants (such as TLR7 activation[40]) and is capable of enhancing T-cell immunity to viral challenge. Evidence suggests that, by interacting with lymphocytes, platelets may facilitate the recruitment of these cells to an injured vessel at a site of inflammation or infection[59], and this is a central step in T-cell trafficking. Activated platelets may mediate T-cell functions further by releasing a number of factors, such as platelet factor 4 (PF4, CXCL4)-RANTES or serotonin[27, 28, 60]. The release of these factors impacts T-cell function in various ways. PF4, for instance, is necessary for the limitation of Th17 expansion and differentiation[61]. Serotonin, the largest peripheral circulation reservoir of which is stored in platelet δ-granules, can push naïve T-cells to activate and proliferate[27, 28].

Platelets and dendritic cells

As previously mentioned, platelets interact with and influence another important cell in adaptive immunity, the DC. Platelets, through the CD40-CD154 axis, can induce DCs to present antigen to T-cells[5]. Additionally, platelet-DC interactions can be mediated by the P-selectin/PSGL1 axis, followed by firm adhesion through platelet junctional adhesion molecule C (JAM-C) with MAC-1 on DCs[62] (Figure 2). Interestingly, this recruitment and activation of DCs is followed by phagocytosis of platelets by DCs[62]. The mechanism by which DCs change after platelet phagocytosis is still largely unknown. It is possible also that serotonin originating from phagocytosed platelets may be utilized by DCs and released as needed for T-cell proliferation and differentiation.

Platelets and natural killer cells

The interaction between platelets and natural killer (NK) cells is the least understood interaction within the circulation (Figure 2). In murine studies, tumors coated with platelets become impermeable to NK cell destruction[63] with fibrinogen involvement[64]. It has been proposed that circulating metastatic cells attract platelets and influence them to release their granule content. As a result, human NK cells lose their cytotoxicity and their ability to produce IFNγ, possibly through the downregulation of NKG2DL mediated by platelet-TGFβ release[65]. However, the baseline communication between platelets and NK cells remains unknown as does the effect on this interaction during infection or autoimmune disease.

Platelet-leukocyte interactions in atherogenesis

The role of platelet-leukocyte interactions during inflammation has been determined to be contributory in murine models of atherosclerosis. It is now established that activated platelets can increase endothelial atherogenic potential and lead to elevated platelet-leukocyte aggregate formations in the circulation. Platelets may further contribute to

atherogenesis through the delivery of proinflammatory factors to leukocytes and endothelial cells[66]. The platelet-derived factors implicated in this setting are surface P-selectin and the platelet-derived chemokines RANTES and PF4[66]. PF4/RANTES heterodimer disruption reduces atherosclerosis in ApoE-deficient mice by attenuating monocyte recruitment[60]. Murine models lacking P-selectin show that this adhesion receptor is a key mediator of leukocyte recruitment into lesions and promotes their advancement in ApoE-deficient mice[67]. In this case, both platelet and endothelial P-selectin contribute to lesion development; however, endothelial P-selectin plays a crucial role in atherosclerotic lesion growth[68]. Similarly, murine models lacking CD40 in ApoE-deficient mice demonstrate that platelet CD40 promotes atherosclerosis by activation and recruitment of leukocytes to endothelial cells[69]. When platelets lacking CD40 are injected in the circulation of mice, expression of VCAM1, PECAM, VE-Cadherin and P-selectin are decreased on endothelial cells. This decrease in adhesion molecule expression is accompanied by less advanced atherosclerotic plaques with reduced levels of macrophages and neutrophils[69]. Increased expression of platelet CD154, in turn, contributes to accelerated atherosclerosis by increasing platelet-platelet interactions and inflammatory response through mediation of platelet-endothelium and platelet-leukocyte interactions[70]. Additionally, platelet CD154 suppresses Treg cell recruitment leading to accelerated atherosclerosis[70].

Platelets and metastatic cells in the circulation

Platelets contribute to the increased thrombotic risk of cancer patients. High platelet count is associated with decreased survival in various malignancies and thrombocytopenia, in turn, is associated with decreased metastatic potential of tumors. Entry of metastatic cancer cells into the blood stream leads to platelet activation. Metastatic cells express PSGL1 and secrete high levels of tissue factor[71]. Platelet-cancer cell interactions and cancer-induced thrombi formation in the vasculature may inhibit recognition and elimination by the immune system. Additionally, the thrombi formation can lead to tethering of the cancer cell to the endothelium, promoting survival and invasive potential. More recent studies in mice have proposed that, as a consequence of interaction with tumor cells, platelet signals recruit granulocytes to the tumor, thereby, guiding the formation of an early prometastatic microenvironment. Depletion of platelets by cell-specific depleting antibodies inhibits granulocyte recruitment to metastatic cells in lungs and depletion of granulocytes leads to fewer metastases and reduced metastatic progression[72].

Platelet-derived TGF-β is an important factor contributing to metastasis. *In vitro* studies have shown that thrombin-mediated platelet-derived TGF-β can alter NK cell antitumor reactivity[65]. *In vivo* studies eliminating TGF-β specifically from platelets have proposed that this cytokine, when originating from platelets, is solely responsible for inducing an invasive epithelial mesenchymal transition to metastasis[73]. These studies utilized the PF4Cre murine model which, at the time, was believed to eliminate TGF-β only from the megakaryocyte/platelet lineage[73]. We have since learned that a subset of epithelial cells can express PF4[74] in addition to activated CD4+ and CD8+ T-cells[61]. These studies suggest that other tissue-resident cells or epithelial cells themselves can contribute to the invasive potential of cancer cells mediated by TGF-β. The role of the platelet-mediated increase in metastatic potential through TGF-β is further complicated because thrombin stimulation also leads to the release

of PF4 from platelet α-granules[61]. PF4, in turn, inhibits TGF-β signaling in a SMAD2/3 dependent manner[61]. Regardless of TGF-β signaling, metastatic cancer cells entering the blood stream hijack the hemostatic function of platelets by releasing tissue factor and, consequently, generate thrombin and initiate thrombus formation and neutrophil recruitment. The formed thrombi can attach to the endothelium and promote viability and metastatic potential of the cancer cell.

Platelets in bacterial infections

During bacterial infections, platelets actively mediate the host response through interactions with circulating leukocytes. In addition to PF4 and RANTES, platelet α-granules contain various antimicrobial compounds such as platelet connective tissue activating peptide 3 (CTAP-3), platelet basic protein, thymosin β-4 (Tβ-4), and fibrinopeptide (A and B)[75, 76]. These platelet-derived antimicrobial compounds are known to target various bacterial organisms (Figure 3)[4, 77, 78]. Our understanding of how platelets mediate these interactions in the circulation is still developing.

Platelets can interact with various strains of bacteria including the *Staphylococci family*, *Neisseria gonorrhea*, *Porphyromonas gingivalis*, and *Helicobacter pylori*[77, 79, 80]. Platelets adhere and aggregate around bacteria, typically utilizing their GP2b/3a, FcγRIIa, and IgG receptors in conjunction with fibrinogen or fibronectin[81, 82]. After platelets interact with bacteria, they release antimicrobial compounds contained in their granules. *S. Aureus* alpha toxin mediates release of β-defensin from platelets and, consequently, β-defensin induces NET formation[48]. Released PF4 can bind to Gram-negative bacteria, leading to the exposure of PF4 heparin-like epitopes. Exposed PF4-epitopes, in turn, increase antibody binding to the bacterial surface leading to the opsonization and possibly phagocytosis of bacteria by neutrophils[83, 84]. This mechanism is particularly important since some Gram-negative bacteria, such as *Yersinia pestis*, are poorly recognized by the mammalian TLR4[85, 86]. *In vivo* studies using another Gram-negative bacteria, *Porphyromonas gingivalis*, show that, during infection, platelets interact with neutrophils forming heterotypic aggregates in a TLR2-dependent manner and TLR2 can also promote platelet aggregation[36]. This study provides evidence that, by recognizing bacterial components, platelets can activate platelet thrombotic and/or inflammatory pathways.

Studies using *Lysteria monocytogenes* have recently shed light on the role of platelets in inducing adaptive immunity during bacterial infection. Platelet binding to intravascular bacteria leads to slowing of the cytotoxic clearance of the pathogen, keeping bacteria available for induction of an adaptive immune response by splenic CD8+ T-cells[87]. This platelet-mediated process is dependent on the opsonization of bacteria by complement C3, subsequent platelet binding by GP1b, and the capture of the bacteria-platelet aggregates by cells of the immune system[87]. Platelet-bacteria interactions in the presence of C3 lead to different kinetics of clearance between free (fast clearance) and platelet-bound (slow clearance) bacteria. This observation is true for Gram-positive strains such as *L. monocytogenes, Bacillus subtilis, Enterococcus faecalis* and *Staphylococcus epidermidis*, and for Gram-negative strains such as *Escherichia coli, Salmonella typhimurium* and

Klebsiella pneumoniae[87]. This study suggests a sophisticated role for platelets in balancing clearance of bacteria and induction of adaptive immunity.

Platelets in viral infections

Platelets interact with various types of viruses. Viral infections are often associated with thrombocytopenia and in some cases thrombosis. Viruses can be simply classified as those with a DNA or RNA genome. RNA viruses are further subdivided into double-stranded and single-stranded viruses. DNA viruses from the herpes viral family such as herpes simplex virus type 1 (HSV1), cytomegalovirus (CMV) and vaccinia, have been found associated with platelets, but it is unclear if they can be internalized by platelets[88–90]. RNA viruses such as HIV, hepatitis C virus (HCV), dengue, influenza, coxsackievirus B (CVB) and encephalomyocarditis virus (EMCV) are much smaller in size and can be internalized by platelets[40, 91–95]. Here, we have focused on the interaction of virally-activated platelets with circulating cells.

In general, platelet function during viral infection can be beneficial and/or detrimental to the host depending on the length of time post infection. Human CMV (hCMV) binds to platelet-TLR2, resulting in release of CD154, IL-1β, and VEGF (Figure 3). hCMV-activated platelets do not show increased aggregation or adhesive potential but rapidly form platelet-neutrophil heterotypic aggregates. In addition to their interactions with neutrophils, hCMV-platelets also exhibit increased interaction with monocytes, B-cells, T-cells and DCs, suggesting a multifactorial interaction with the innate and adaptive immune systems and induction of proangiogenic responses[88]. *In vitro* studies utilizing vaccinia virus show that ADP-, collagen-, or thrombin-mediated aggregation in isolated platelets is inhibited by the virus but there is an increase in serotonin release[90] (Figure 3). *In vivo* studies, however, show that infection with vaccinia leads to fatal intravascular coagulation at 24h post infection[96]. *In vitro* studies using HSV1-infected human umbilical vein endothelial cells (HUVEC) show platelet binding due to increased thrombin generation from the damaged cells[97]. These observations suggest that other circulating or vascular cells can contribute to the overall prothrombotic balance.

RNA virus infections exhibit more diverse platelet responses. Many RNA viruses cause various degrees of thrombocytopenia at different stages of infection. In the setting of murine EMCV infection, platelets interact with neutrophils to form heterotypic aggregates as early as 1h post infection and this leads to a sudden drop in platelet count. This drop also occurs at 24h post infection. Elimination of platelets before EMCV infection leads to reduced survival similar to mice deficient in TLR7[40].

In humans, infection with dengue, an RNA virus, is characterized by profound hemorrhagic fever and thrombocytopenia. In a rhesus macaque model, dengue viral infection leads to platelet-monocyte interactions at 24h post infection while platelet-neutrophil interactions peak at 3d post infection[98]. In this study, blood contained monocytes that had engulfed platelets containing dengue virus[98]. Increased permeability of the endothelium during dengue infection is also facilitated by platelets. Dengue infection mediates assembly of the NLPR3 inflammasome and activation of Caspase-1 in platelets. Caspase-1, in turn, alters

secretion of IL-1β-rich microparticles[99]. Platelet-monocyte interactions are also increased in people infected with HIV1 or influenza[100, 101] and are influenced by inflammatory stimuli in a COX-dependent manner[102].

During infection with CVB, platelets form aggregates with the neutrophil population and directly improve the outcome of CVB-induced myocarditis[95]. CVB is internalized by platelets causing phosphatidylserine exposure on the platelet surface without affecting apoptosis[95]. The virus cannot replicate in platelets and platelet depletion causes an increased viral load in heart tissue as well as increased myocarditis[95].

Influenza infection illustrates the potential for dysregulation of the hemostatic-inflammatory balance. In patients infected with influenza H1N1 virus, there is an elevation of platelet-monocyte aggregates and a two-fold increase in platelet aggregation potential as measured by PAC1 incorporation[103] (Figure 3). As the infection progresses, coagulation potential increases, as evidenced by the elevation of macrovesicle tissue factor activity[104]. In addition, in non-survivor influenza-infected patients, there is a trend toward lowered platelet and increased white blood cell counts[104]. H1N1 activates human platelets independently through both FcγRIIA signaling and through thrombin generation[105], while H1N1–platelet interaction increases the expression of the active form of the fibrinogen receptor GP2b/3a, arachidonic acid metabolism, and microparticle release[105]. Studies utilizing a lethal dose of the H1N1/PR8 strain in C57BL/6J mice suggest that, in critical non-survivor settings, platelet degranulation and activation contribute to lung inflammation and reduced survival[106]. In summary, platelets contribute to the overall procoagulant and inflammatory states during influenza infection, but it is unclear if they are necessary for overall immunity and vascular health.

Platelets in Plasmodium parasite (malaria) infection

There are six plasmodium species that can infect humans and lead to development of malaria. The role of platelets in *Plasmodium* parasite infection changes as the infection progresses. Studies using non-inflammatory malaria with *P. falciparum* or *P. chabaudi* show that platelets kill the parasite inside of the red blood cells (RBCs) by secreting PF4[107]. PF4, in turn, interacts with the Duffy antigen on RBCs through an unknown mechanism and mediates survival of infection[108].

Although these studies suggest a protective role during early infection, a model of cerebral malaria using *P. berghei* ANKA (PbA) iRBCs clone 2.34 concludes that platelets play multifactorial roles depending on the pathogenic stage of infection[109]. Platelets in this case were activated throughout the first 3 days of infection as measured by an increase in circulating PF4. This early activation of platelets is protective possibly due to a platelet-dependent reduction in parasite burden and leukocyte recruitment through an increase of the IL-1β pool[109]. As infection progresses, however, platelet activation, possibly mediated by the overall immune response and tissue factor release, leads to microvascular thrombosis as found in the postmortem brains of patients with cerebral malaria[4,110].

Platelets and RNA transfer

The ability of platelets to communicate with other vascular cells through receptor signaling, direct protein interactions, or released granule content has long been understood. More recently, other methods of platelet vascular communication have been described including RNA transfer. Bidirectional RNA transmission is illustrated by transfer of platelet-derived transcripts to leukocytes or endothelial cells and by the uptake of leukocyte- or vascular-derived transcripts into platelets[111] (Figure 4). Co-culture of platelet-like particles (PLPs) with either a HUVEC cell line or a human monocytic cell line (THP-1) demonstrates functional transfer of platelet-derived mRNA transcripts[111]. Endogenous analysis of THP-1 cells post PLP incubation reveals an increased expression of α, γ, and ε globin genes as a consequence of RNA transfer[111]. Confirmation of this phenomenon *in vivo* utilizing mouse infusion experiments demonstrates that platelets carrying TLR2 mRNA can transfer their transcripts to mice with TLR2-deficient blood mononuclear leukocytes[111].

Analysis of human platelets post co-incubation with HUVECs and THP-1 cells reveals that platelets can also take up transcripts from surrounding cells. There are distinct changes in expression of platelet transcripts including those associated with platelet-vascular adhesion and apoptosis[112]. Platelet RNA uptake has been confirmed by the presence of TLR2 in TLR2-negative platelets post infusion of TLR2-positive leukocytes (peripheral blood mononuclear cells, PBMCs) into TLR2 knockout mice[112]. Interestingly, platelet RNA uptake can occur in the presence or absence of thrombin stimulation[112].

Platelet microparticles, miRNA and vascular inflammation

In addition to the hemostatic and inflammatory responses described, platelet activation also leads to microparticle formation. This is a particularly important observation since platelets are a major source of microparticles in the circulation[4]. Proteins that mediate platelet function related to hemostasis or immunity are also found in platelet microparticles. This includes adhesion molecules such as P-selectin, chemokines such as RANTES, and cytokines such as IL-1β, among others[99, 113]. Platelet microparticles can promote monocyte recruitment to the inflamed endothelium of the atherosclerotic plaque[113]. Increased circulating platelet microparticles have been associated with atherosclerosis development in diabetic patients. Infections such as influenza or dengue also lead to increased platelet microparticle release[99]. During vascular injury, platelet microparticles can increase the regenerative potential of endothelial progenitor cells by enhancing their recruitment, differentiation and release of proangiogenic factors[114].

In addition to proteins, platelet microparticles contain various forms of RNA including micro RNA (miRNA), which are small non-coding RNAs that function in post-transcriptional regulation of gene expression. Platelets may affect surrounding cells by transferring miRNA[115,116]. The platelet miRNAs, miR-223 or miR-320b, are present in platelet-derived microparticles post thrombin stimulation and can transfer into endothelial cells[117, 115] (Figure 4). Functionally, it has been shown that miR-320b transfer into endothelial cells can decrease ICAM1 expression[115]. Similarly, miR-126-3p, highly expressed in platelets, can be taken up and enriched in macrophages, resulting in an

increased phagocytic phenotype[116]. Platelet microparticle-derived miR-223 transfer to cancer cells may play a role in cancer progression. This miRNA transfer leads to downregulation of the tumor suppressor EPB4L13 and a concurrent increase in tumor cell invasion[118]. Alternatively, transfer of platelet-derived miR-24 to solid tumor cells results in disrupted mitochondrial function, leading to tumor cell death and proliferation decline[119]. These studies support the hypothesis that platelet RNA can affect function of vascular or circulating cells through RNA transfer.

In support of the relevance of miRNA transfer, is the observation of decreased miRNA expression only in platelets at the site of injury and not in the peripheral circulation[115]. While the differential expression of miRNAs in platelet-derived microparticles is interesting, it is crucial to establish how these microparticle miRNAs affect the function of recipient cells.

Platelets and mitochondrial transfer

As both protein and RNA communication between platelets and the vasculature can occur through microparticle production, there is the possibility that other platelet contents may transfer. Platelets contain mitochondria (averaging four mitochondria/platelet) that are both functional and have been shown to impact platelet activation and subsequent thrombosis[44, 120, 121]. Platelet-derived mitochondria may play a role in communication as platelets release functional mitochondria when stimulated with numerous agonists via both platelet microparticles and as extracellular free organelles[44]. These mitochondria may impact overall vascular health and level of inflammation[44]. Extracellular mitochondria have been implicated as damage-associated molecular patterns (DAMPs)[44, 122–125]. Additionally, mitochondria can act as a substrate for sPLA2-IIA, a phospholipase known to be induced during inflammatory settings[44]. In its interaction with extracellular mitochondria, sPLA2-IIA leads to mitochondrial dysfunction and subsequent mitochondrial content release (arachidonic acid, lysophospholipids and mitochondrial DNA), all of which reinforce a proinflammatory environment. sPLA2-IIA has also been implicated in the internalization of platelet microparticles by neutrophils[44] (Figure 4). Released mitochondria interact and are internalized by neutrophils, where mitochondria-neutrophil interactions appear to impact neutrophil functionality (increased rolling and interaction with vascular walls, increased induction of NETs), morphology (development of pseudopodia), and substance release (leukotrienes)[44]. Finally, extracellular mitochondria expression is linked to a number of proinflammatory settings. Mouse studies show that sPLA2-IIA-dependent release of mitochondrial factors leads to increased proinflammatory cytokine release and decreased body temperature[44]. Human studies reveal increased levels of extracellular mitochondria in the synovial fluid of multiple inflammatory autoimmune pathologies such as rheumatoid arthritis and osteoarthritis[44], and these levels correlate with the known elevation in platelet microparticles[44, 126–128]. These observations suggest that extracellularly released platelet mitochondria may function as a potent communicator of vascular inflammation.

Antiplatelet therapies and complex platelet function

In addition to continual use of established platelet inhibitors, there has been great progress in the development of antiplatelet/antithrombotic therapies for the treatment of various thrombotic pathologies. Cyclooxygenase (COX) in platelets, responsible for TxA2 generation and three dimensional clot formation, has been targeted in the regulation of platelet activity. Platelets contain predominantly COX1 while endothelial cells can contain COX1 and COX2. Aspirin is an irreversible inhibitor of COX activity, and as platelets do not regenerate COX protein, this drug is an effective inhibitor of platelet aggregation potential. Aspirin has provided beneficial effective secondary prevention of arterial thromboembolic events.[35] Inhibition of the prothrombotic activity of platelets by aspirin is also considered to be beneficial in infectious diseases such as influenza. Relevant to the role of platelets in cancer, it is suggested that aspirin may be beneficial in preventing the platelet microthrombi formation around cancer cells in the circulation that may aid metastasis. A recent meta-analysis study concludes that long term aspirin use is associated with reduced risk of overall cancer[129].

A range of P2Y12 inhibitors are also extensively used in patients with cardiovascular syndromes. *In vitro* blocking of P2Y12 by Cangrelor reduces platelet deposition on the injured endothelium and delays vessel sealing, thereby leading to prolonged escape of plasma-born molecules into the microvaculature[130]. The results observed with inhibition of GP2b/3a signaling are more complex. While still, on occasion, used intravenously to inhibit platelet function in the setting of acute unstable cardiac ischemia[14], oral formulations were associated with increased morbidity and mortality[131]. Still unknown is whether inhibiting P2Y12 or GP2b/3a will alter the immune responding functions of the platelet.

Interestingly, the thrombin inhibitor, hirudin, which binds and inhibits thrombin and prevents fibrin generation, does not block the sealing of vessel injury[130]. This observation suggests that the core and the shell of clots can form without fibrin but the stability and retraction may be affected. Finally, TLR agonists/antagonists may be useful in promoting platelet immune function and survival of infection possibly depending on the specific receptor. This is consistent with the observation that elimination of platelets before infection with the TLR7-activating virus, EMCV, leads to decreased survival of mice[40]. As therapeutics designed to target platelets progress, the type of injury, mechanism/type of infection, and timing of infection must be considered.

Conclusion

Platelets exhibit complex interactions with circulating cells and the vessel wall during a broad range of inflammatory processes that manifest in many types of disease. However, there is overlap between the platelets' hemostatic and inflammatory roles. For example, platelets may engage leukocytes and the immune system by utilizing proteins involved in hemostatic plug formation. More recent data suggest that these interactions are regulated by processes beyond traditionally-defined receptors, e.g. RNA or mitochondrial transfer to other vascular or circulating cells. These complex and sometimes contradictory interactions appear to directly affect disease. During certain stages of infection, platelets initiate

activation of both innate and adaptive immunity which is beneficial to the host. However, uncontrolled endothelial damage and inflammation as a result of viral infection progression can lead to adverse prothrombotic responses and increased cardiovascular risk. Thus, platelets appear to have broad and multifaceted functions firmly grounded in their vascular cell interactions. Maintaining these complicated and distinct states of activation is imperative to regulating vascular homeostasis and health. As our understanding of the role of platelets in the vasculature and circulation grows, we have the potential to design better platelet-targeted treatments for patients who present with a wide range of vascular, immune, and oncological diseases.

Acknowledgments

We apologize to all whose work was not cited due to limited space and limited number of citations.

Sources of Funding:

This work was supported by AHA Award 16SDG30450001 (to M.K.) and U54 HL12311, NIH Common Fund Award UH3 TR000921, and AHA Award 16SFRN31740000 (to J.E.F.).

Nonstandard Abbreviations and Acronyms

VTE	venous thromboembolism
GP	glycoprotein GP2b/3a
vWF	von Willebrand factor
TxA2	thromboxane
MI	myocardial infarction
P-selectin	platelet selectin
E-selectin	endothelial selectin
PSGL1	P-Selectin glycoprotein ligand 1
PAR4	protease-activated receptor-4
PF4	platelet factor 4 (also CXCL4)
CXCL7	Chemokine (C-X-C motif) ligand 7
CXCR1/2	C-X-C Chemokine Receptor Type 1 or 2
CX3CR1	C-X3-C Motif Chemokine Receptor 1
ICAM1	Intercellular Adhesion Molecule 1
VCAM1	vascular cell adhesion molecule 1
PECAM	Platelet endothelial cell adhesion molecule (also CD31)
VE-cadherin	vascular endothelial cadherin

NO	nitric oxide
IL-1β	Interleukin 1 beta
IL-8	Interleukin 8
IL-6	Interleukin 6
MCP-1	Monocyte chemotactic protein 1 (also CCL2)
TLR	toll-like receptor
RANTES	regulated on activation, normal T cell expressed and secreted (also CCL5)
DCs	dendritic cells
NK	natural killer cells
TNFRII	Tumor necrosis factor receptor 2
HNP1	human neutrophil peptide 1
GM-CSF	granulocyte-macrophage colony-stimulating factor
LPS	Lipopolysaccharide
IgG	Immunoglobulin G
JAM-C	junctional adhesion molecule C
MAC-1	Macrophage-1 antigen (also integrin $\alpha_M\beta_2$ or CD11b/CD18)
IFNγ	interferon gamma
TGFβ	Transforming growth factor beta
NKG2DL	natural killer G2D ligand
ApoE	apolipoprotein E
CTAP-3	connective tissue activating peptide 3
Tβ-4	thymosin β-4
FcγRIIa	Fc fragment of IgG receptor IIa
NET	neutrophil extracellular trap
hCMV	human cytomegalovirus
HSV	herpes simplex virus
CVB	coxsackievirus B
EMCV	encephalomyocarditis virus

HCV	hepatitis C virus
VEGF	vascular endothelial growth factor
HUVEC	human umbilical vein endothelial cells
COX	Cyclooxygenase
PAC-1	antibody clone PAC-1 recognizing an epitope on GP2b/3a and measures platelet activation
PLP	platelet like particles
PBMCs	peripheral blood mononuclear cells
sPLA2-IIA	Serum secretory phospholipase A2-IIa
P2Y12	purinergic receptor 2Y12

References

1. Michelson, AD. Platelets. London ; Waltham, MA: Academic Press; 2013.
2. Ghoshal K, Bhattacharyya M. Overview of platelet physiology: Its hemostatic and nonhemostatic role in disease pathogenesis. ScientificWorldJournal. 2014; 2014:781857. [PubMed: 24729754]
3. Drelich, DA., Bray, PF. The traditional role of platelets in hemostasis. In: Kerrigan, S., Moran, N., editors. The non-thrombotic role of platelets in health and disease. Rijeka: InTech; 2015. Ch. 02
4. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. Blood. 2014; 123:2759–2767. [PubMed: 24585776]
5. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. Nat Rev Immunol. 2011; 11:264–274. [PubMed: 21436837]
6. Koupenova M, Kehrel BE, Corkrey HA, Freedman JE. Thrombosis and platelets: An update. Eur Heart J. 2017; 38:785–791. [PubMed: 28039338]
7. Ciferri S, Emiliani C, Guglielmini G, Orlacchio A, Nenci GG, Gresele P. Platelets release their lysosomal content in vivo in humans upon activation. Thromb Haemost. 2000; 83:157–164. [PubMed: 10669170]
8. Jurk K, Kehrel BE. Platelets: Physiology and biochemistry. Semin Thromb Hemost. 2005; 31:381–392. [PubMed: 16149014]
9. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol. 2013; 13:34–45. [PubMed: 23222502]
10. Tichelaar YI, Kluin-Nelemans HJ, Meijer K. Infections and inflammatory diseases as risk factors for venous thrombosis. A systematic review. Thromb Haemost. 2012; 107:827–837. [PubMed: 22437808]
11. Bunce PE, High SM, Nadjafi M, Stanley K, Liles WC, Christian MD. Pandemic h1n1 influenza infection and vascular thrombosis. Clin Infect Dis. 2011; 52:e14–17. [PubMed: 21288835]
12. Campbell LA, Rosenfeld ME. Infection and atherosclerosis development. Arch Med Res. 2015; 46:339–350. [PubMed: 26004263]
13. Stalker TJ, Traxler EA, Wu J, Wannemacher KM, Cermignano SL, Voronov R, Diamond SL, Brass LF. Hierarchical organization in the hemostatic response and its relationship to the platelet-signaling network. Blood. 2013; 121:1875–1885. [PubMed: 23303817]
14. Tomaiuolo M, Brass LF, Stalker TJ. Regulation of platelet activation and coagulation and its role in vascular injury and arterial thrombosis. Interv Cardiol Clin. 2017; 6:1–12. [PubMed: 27886814]
15. Kaplan ZS, Zarpellon A, Alwis I, Yuan Y, McFadyen J, Ghasemzadeh M, Schoenwaelder SM, Ruggeri ZM, Jackson SP. Thrombin-dependent intravascular leukocyte trafficking regulated by fibrin and the platelet receptors gpib and par4. Nat Commun. 2015; 6:7835. [PubMed: 26204458]

16. Ghasemzadeh M, Kaplan ZS, Alwis I, Schoenwaelder SM, Ashworth KJ, Westein E, Hosseini E, Salem HH, Slattery R, McColl SR, Hickey MJ, Ruggeri ZM, Yuan Y, Jackson SP. The cxcr1/2 ligand nap-2 promotes directed intravascular leukocyte migration through platelet thrombi. Blood. 2013; 121:4555–4566. [PubMed: 23550035]
17. Etulain J, Schattner M. Glycobiology of platelet-endothelial cell interactions. Glycobiology. 2014; 24:1252–1259. [PubMed: 24928621]
18. Wagner DD, Frenette PS. The vessel wall and its interactions. Blood. 2008; 111:5271–5281. [PubMed: 18502843]
19. Khlgatian M, Nassar H, Chou HH, Gibson FC 3rd, Genco CA. Fimbria-dependent activation of cell adhesion molecule expression in porphyromonas gingivalis-infected endothelial cells. Infect Immun. 2002; 70:257–267. [PubMed: 11748191]
20. Gkaliagkousi E, Ritter J, Ferro A. Platelet-derived nitric oxide signaling and regulation. Circ Res. 2007; 101:654–662. [PubMed: 17901370]
21. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, Kroczek RA. Cd40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature. 1998; 391:591–594. [PubMed: 9468137]
22. Denis MM, Tolley ND, Bunting M, Schwertz H, Jiang H, Lindemann S, Yost CC, Rubner FJ, Albertine KH, Swoboda KJ, Fratto CM, Tolley E, Kraiss LW, McIntyre TM, Zimmerman GA, Weyrich AS. Escaping the nuclear confines: Signal-dependent pre-mrna splicing in anucleate platelets. Cell. 2005; 122:379–391. [PubMed: 16096058]
23. Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, Zimmerman GA, Weyrich AS. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. J Cell Biol. 2001; 154:485–490. [PubMed: 11489912]
24. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol. 2007; 7:803–815. [PubMed: 17893694]
25. Puhlmann M, Weinreich DM, Farma JM, Carroll NM, Turner EM, Alexander HR Jr. Interleukin-1beta induced vascular permeability is dependent on induction of endothelial tissue factor (tf) activity. J Transl Med. 2005; 3:37. [PubMed: 16197553]
26. Beaulieu LM, Lin E, Mick E, Koupenova M, Weinberg EO, Kramer CD, Genco CA, Tanriverdi K, Larson MG, Benjamin EJ, Freedman JE. Interleukin 1 receptor 1 and interleukin 1beta regulate megakaryocyte maturation, platelet activation, and transcript profile during inflammation in mice and humans. Arterioscler Thromb Vasc Biol. 2014; 34:552–564. [PubMed: 24458711]
27. Leon-Ponte M, Ahern GP, O'Connell PJ. Serotonin provides an accessory signal to enhance t-cell activation by signaling through the 5-ht7 receptor. Blood. 2007; 109:3139–3146. [PubMed: 17158224]
28. O'Connell PJ, Wang X, Leon-Ponte M, Griffiths C, Pingle SC, Ahern GP. A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and t cells. Blood. 2006; 107:1010–1017. [PubMed: 16223770]
29. Crawford A, Angelosanto JM, Nadwodny KL, Blackburn SD, Wherry EJ. A role for the chemokine rantes in regulating cd8 t cell responses during chronic viral infection. PLoS Pathog. 2011; 7:e1002098. [PubMed: 21814510]
30. Koupenova M, Mick E, Mikhalev E, Benjamin EJ, Tanriverdi K, Freedman JE. Sex differences in platelet toll-like receptors and their association with cardiovascular risk factors. Arterioscler Thromb Vasc Biol. 2015; 35:1030–1037. [PubMed: 25657311]
31. Cognasse F, Nguyen KA, Damien P, McNicol A, Pozzetto B, Hamzeh-Cognasse H, Garraud O. The inflammatory role of platelets via their tlrs and siglec receptors. Front Immunol. 2015; 6:83. [PubMed: 25784910]
32. Nocella C, Carnevale R, Bartimoccia S, Novo M, Cangemi R, Pastori D, Calvieri C, Pignatelli P, Violi F. Lipopolysaccharide as trigger of platelet aggregation via eicosanoid over-production. Thromb Haemost. 2017; 117:1558–1570. [PubMed: 28492699]
33. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, Devinney R, Doig CJ, Green FH, Kubes P. Platelet tlr4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med. 2007; 13:463–469. [PubMed: 17384648]

34. Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional toll-like receptor-4. Blood. 2005; 106:2417–2423. [PubMed: 15961512]

35. Biswas S, Zimman A, Gao D, Byzova T, Podrez E. Tlr2 plays a key role in platelet hyperreactivity and accelerated thrombosis associated with hyperlipidemia. Circ Res. 2017

36. Blair P, Rex S, Vitseva O, Beaulieu L, Tanriverdi K, Chakrabarti S, Hayashi C, Genco CA, Iafrati M, Freedman JE. Stimulation of toll-like receptor 2 in human platelets induces a thromboinflammatory response through activation of phosphoinositide 3-kinase. Circ Res. 2009; 104:346–354. [PubMed: 19106411]

37. Falker K, Klarstrom-Engstrom K, Bengtsson T, Lindahl TL, Grenegard M. The toll-like receptor 2/1 (tlr2/1) complex initiates human platelet activation via the src/syk/lat/plcgamma2 signalling cascade. Cell Signal. 2014; 26:279–286. [PubMed: 24240055]

38. Panigrahi S, Ma Y, Hong L, Gao D, West XZ, Salomon RG, Byzova TV, Podrez EA. Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyperreactivity and thrombosis. Circ Res. 2013; 112:103–112. [PubMed: 23071157]

39. Thon JN, Peters CG, Machlus KR, Aslam R, Rowley J, Macleod H, Devine MT, Fuchs TA, Weyrich AS, Semple JW, Flaumenhaft R, Italiano JE Jr. T granules in human platelets function in tlr9 organization and signaling. J Cell Biol. 2012; 198:561–574. [PubMed: 22908309]

40. Koupenova M, Vitseva O, MacKay CR, Beaulieu LM, Benjamin EJ, Mick E, Kurt-Jones EA, Ravid K, Freedman JE. Platelet-tlr7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. Blood. 2014; 124:791–802. [PubMed: 24755410]

41. D'Atri LP, Etulain J, Rivadeneyra L, Lapponi MJ, Centurion M, Cheng K, Yin H, Schattner M. Expression and functionality of toll-like receptor 3 in the megakaryocytic lineage. J Thromb Haemost. 2015; 13:839–850. [PubMed: 25594115]

42. Anabel AS, Eduardo PC, Pedro Antonio HC, Carlos SM, Juana NM, Honorio TA, Nicolas VS, Sergio Roberto AR. Human platelets express toll-like receptor 3 and respond to poly i:C. Hum Immunol. 2014; 75:1244–1251. [PubMed: 25315747]

43. Maugeri N, Rovere-Querini P, Evangelista V, Covino C, Capobianco A, Bertilaccio MT, Piccoli A, Totani L, Cianflone D, Maseri A, Manfredi AA. Neutrophils phagocytose activated platelets in vivo: A phosphatidylserine, p-selectin, and {beta}2 integrin-dependent cell clearance program. Blood. 2009; 113:5254–5265. [PubMed: 19264679]

44. Boudreau LH, Duchez AC, Cloutier N, Soulet D, Martin N, Bollinger J, Pare A, Rousseau M, Naika GS, Levesque T, Laflamme C, Marcoux G, Lambeau G, Farndale RW, Pouliot M, Hamzeh-Cognasse H, Cognasse F, Garraud O, Nigrovic PA, Guderley H, Lacroix S, Thibault L, Semple JW, Gelb MH, Boilard E. Platelets release mitochondria serving as substrate for bactericidal group iia-secreted phospholipase a2 to promote inflammation. Blood. 2014; 124:2173–2183. [PubMed: 25082876]

45. Jenne CN, Kubes P. Virus-induced nets--critical component of host defense or pathogenic mediator? PLoS Pathog. 2015; 11:e1004546. [PubMed: 25569217]

46. Martinod K, Wagner DD. Thrombosis: Tangled up in nets. Blood. 2014; 123:2768–2776. [PubMed: 24366358]

47. Kaplan MJ, Radic M. Neutrophil extracellular traps: Double-edged swords of innate immunity. J Immunol. 2012; 189:2689–2695. [PubMed: 22956760]

48. Kraemer BF, Campbell RA, Schwertz H, Cody MJ, Franks Z, Tolley ND, Kahr WH, Lindemann S, Seizer P, Yost CC, Zimmerman GA, Weyrich AS. Novel anti-bacterial activities of beta-defensin 1 in human platelets: Suppression of pathogen growth and signaling of neutrophil extracellular trap formation. PLoS Pathog. 2011; 7:e1002355. [PubMed: 22102811]

49. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface p-selectin: Studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation. 2001; 104:1533–1537. [PubMed: 11571248]

50. Sarma J, Laan CA, Alam S, Jha A, Fox KA, Dransfield I. Increased platelet binding to circulating monocytes in acute coronary syndromes. Circulation. 2002; 105:2166–2171. [PubMed: 11994250]

51. Furman MI, Barnard MR, Krueger LA, Fox ML, Shilale EA, Lessard DM, Marchese P, Frelinger AL 3rd, Goldberg RJ, Michelson AD. Circulating monocyte-platelet aggregates are an early

marker of acute myocardial infarction. J Am Coll Cardiol. 2001; 38:1002–1006. [PubMed: 11583872]

52. Shih L, Kaplan D, Kraiss LW, Casper TC, Pendleton RC, Peters CL, Supiano MA, Zimmerman GA, Weyrich AS, Rondina MT. Platelet-monocyte aggregates and c-reactive protein are associated with vte in older surgical patients. Sci Rep. 2016; 6:27478. [PubMed: 27270163]
53. Alard JE, Ortega-Gomez A, Wichapong K, Bongiovanni D, Horckmans M, Megens RT, Leoni G, Ferraro B, Rossaint J, Paulin N, Ng J, Ippel H, Suylen D, Hinkel R, Blanchet X, Gaillard F, D'Amico M, von Hundelshausen P, Zarbock A, Scheiermann C, Hackeng TM, Steffens S, Kupatt C, Nicolaes GA, Weber C, Soehnlein O. Recruitment of classical monocytes can be inhibited by disturbing heteromers of neutrophil hnp1 and platelet ccl5. Sci Transl Med. 2015; 7:317ra196.
54. Postea O, Vasina EM, Cauwenberghs S, Projahn D, Liehn EA, Lievens D, Theelen W, Kramp BK, Butoi ED, Soehnlein O, Heemskerk JW, Ludwig A, Weber C, Koenen RR. Contribution of platelet cx(3)cr1 to platelet-monocyte complex formation and vascular recruitment during hyperlipidemia. Arterioscler Thromb Vasc Biol. 2012; 32:1186–1193. [PubMed: 22383701]
55. Raiden S, Schettini J, Salamone G, Trevani A, Vermeulen M, Gamberale R, Giordano M, Geffner J. Human platelets produce granulocyte-macrophage colony-stimulating factor and delay eosinophil apoptosis. Lab Invest. 2003; 83:589–598. [PubMed: 12695562]
56. Pitchford SC, Momi S, Giannini S, Casali L, Spina D, Page CP, Gresele P. Platelet p-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. Blood. 2005; 105:2074–2081. [PubMed: 15528309]
57. Li N, Ji Q, Hjemdahl P. Platelet-lymphocyte conjugation differs between lymphocyte subpopulations. J Thromb Haemost. 2006; 4:874–881. [PubMed: 16634758]
58. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Chavarin P, Cogne M, Richard Y, Garraud O. Human platelets can activate peripheral blood b cells and increase production of immunoglobulins. Exp Hematol. 2007; 35:1376–1387. [PubMed: 17656005]
59. Hu H, Zhu L, Huang Z, Ji Q, Chatterjee M, Zhang W, Li N. Platelets enhance lymphocyte adhesion and infiltration into arterial thrombus. Thromb Haemost. 2010; 104:1184–1192. [PubMed: 20838746]
60. Koenen RR, von Hundelshausen P, Nesmelova IV, Zernecke A, Liehn EA, Sarabi A, Kramp BK, Piccinini AM, Paludan SR, Kowalska MA, Kungl AJ, Hackeng TM, Mayo KH, Weber C. Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. Nat Med. 2009; 15:97–103. [PubMed: 19122657]
61. Shi G, Field DJ, Ko KA, Ture S, Srivastava K, Levy S, Kowalska MA, Poncz M, Fowell DJ, Morrell CN. Platelet factor 4 limits th17 differentiation and cardiac allograft rejection. J Clin Invest. 2014; 124:543–552. [PubMed: 24463452]
62. Langer HF, Daub K, Braun G, Schonberger T, May AE, Schaller M, Stein GM, Stellos K, Bueltmann A, Siegel-Axel D, Wendel HP, Aebert H, Roecken M, Seizer P, Santoso S, Wesselborg S, Brossart P, Gawaz M. Platelets recruit human dendritic cells via mac-1/jam-c interaction and modulate dendritic cell function in vitro. Arterioscler Thromb Vasc Biol. 2007; 27:1463–1470. [PubMed: 17379836]
63. Nieswandt B, Hafner M, Echtenacher B, Mannel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. Cancer Res. 1999; 59:1295–1300. [PubMed: 10096562]
64. Palumbo JS, Talmage KE, Massari JV, La Jeunesse CM, Flick MJ, Kombrinck KW, Jirouskova M, Degen JL. Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. Blood. 2005; 105:178–185. [PubMed: 15367435]
65. Kopp HG, Placke T, Salih HR. Platelet-derived transforming growth factor-beta down-regulates nkg2d thereby inhibiting natural killer cell antitumor reactivity. Cancer Res. 2009; 69:7775–7783. [PubMed: 19738039]
66. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, Littman DR, Weber C, Ley K. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein e. Nat Med. 2003; 9:61–67. [PubMed: 12483207]
67. Dong ZM, Brown AA, Wagner DD. Prominent role of p-selectin in the development of advanced atherosclerosis in apoe-deficient mice. Circulation. 2000; 101:2290–2295. [PubMed: 10811597]

68. Burger PC, Wagner DD. Platelet p-selectin facilitates atherosclerotic lesion development. Blood. 2003; 101:2661–2666. [PubMed: 12480714]
69. Gerdes N, Seijkens T, Lievens D, Kuijpers MJ, Winkels H, Projahn D, Hartwig H, Beckers L, Megens RT, Boon L, Noelle RJ, Soehnlein O, Heemskerk JW, Weber C, Lutgens E. Platelet cd40 exacerbates atherosclerosis by transcellular activation of endothelial cells and leukocytes. Arterioscler Thromb Vasc Biol. 2016; 36:482–490. [PubMed: 26821950]
70. Lievens D, Zernecke A, Seijkens T, Soehnlein O, Beckers L, Munnix IC, Wijnands E, Goossens P, van Kruchten R, Thevissen L, Boon L, Flavell RA, Noelle RJ, Gerdes N, Biessen EA, Daemen MJ, Heemskerk JW, Weber C, Lutgens E. Platelet cd40l mediates thrombotic and inflammatory processes in atherosclerosis. Blood. 2010; 116:4317–4327. [PubMed: 20705757]
71. Mueller BM, Reisfeld RA, Edgington TS, Ruf W. Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis. Proc Natl Acad Sci U S A. 1992; 89:11832–11836. [PubMed: 1465406]
72. Labelle M, Begum S, Hynes RO. Platelets guide the formation of early metastatic niches. Proc Natl Acad Sci U S A. 2014; 111:E3053–3061. [PubMed: 25024172]
73. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell. 2011; 20:576–590. [PubMed: 22094253]
74. Pertuy F, Aguilar A, Strassel C, Eckly A, Freund JN, Duluc I, Gachet C, Lanza F, Leon C. Broader expression of the mouse platelet factor 4-cre transgene beyond the megakaryocyte lineage. J Thromb Haemost. 2015; 13:115–125. [PubMed: 25393502]
75. Blair P, Flaumenhaft R. Platelet alpha-granules: Basic biology and clinical correlates. Blood Rev. 2009; 23:177–189. [PubMed: 19450911]
76. Tang YQ, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. Infect Immun. 2002; 70:6524–6533. [PubMed: 12438321]
77. Yeaman MR. Platelets in defense against bacterial pathogens. Cell Mol Life Sci. 2010; 67:525–544. [PubMed: 20013024]
78. Assinger A. Platelets and infection - an emerging role of platelets in viral infection. Front Immunol. 2014; 5:649. [PubMed: 25566260]
79. Fitzgerald JR, Foster TJ, Cox D. The interaction of bacterial pathogens with platelets. Nat Rev Microbiol. 2006; 4:445–457. [PubMed: 16710325]
80. Hamzeh-Cognasse H, Damien P, Chabert A, Pozzetto B, Cognasse F, Garraud O. Platelets and infections - complex interactions with bacteria. Front Immunol. 2015; 6:82. [PubMed: 25767472]
81. Fitzgerald JR, Loughman A, Keane F, Brennan M, Knobel M, Higgins J, Visai L, Speziale P, Cox D, Foster TJ. Fibronectin-binding proteins of staphylococcus aureus mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin gpiib/iiia and igg binding to the fcgammariia receptor. Mol Microbiol. 2006; 59:212–230. [PubMed: 16359330]
82. Loughman A, Fitzgerald JR, Brennan MP, Higgins J, Downer R, Cox D, Foster TJ. Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by staphylococcus aureus clumping factor a. Mol Microbiol. 2005; 57:804–818. [PubMed: 16045623]
83. Krauel K, Weber C, Brandt S, Zahringer U, Mamat U, Greinacher A, Hammerschmidt S. Platelet factor 4 binding to lipid a of gram-negative bacteria exposes pf4/heparin-like epitopes. Blood. 2012; 120:3345–3352. [PubMed: 22942185]
84. Krauel K, Potschke C, Weber C, Kessler W, Furll B, Ittermann T, Maier S, Hammerschmidt S, Broker BM, Greinacher A. Platelet factor 4 binds to bacteria, [corrected] inducing antibodies cross-reacting with the major antigen in heparin-induced thrombocytopenia. Blood. 2011; 117:1370–1378. [PubMed: 20959601]
85. Dziarski R. Deadly plague versus mild-mannered tlr4. Nat Immunol. 2006; 7:1017–1019. [PubMed: 16985495]
86. Montminy SW, Khan N, McGrath S, Walkowicz MJ, Sharp F, Conlon JE, Fukase K, Kusumoto S, Sweet C, Miyake K, Akira S, Cotter RJ, Goguen JD, Lien E. Virulence factors of yersinia pestis are overcome by a strong lipopolysaccharide response. Nat Immunol. 2006; 7:1066–1073. [PubMed: 16980981]

87. Broadley SP, Plaumann A, Coletti R, Lehmann C, Wanisch A, Seidlmeier A, Esser K, Luo S, Ramer PC, Massberg S, Busch DH, van Lookeren Campagne M, Verschoor A. Dual-track clearance of circulating bacteria balances rapid restoration of blood sterility with induction of adaptive immunity. Cell Host Microbe. 2016; 20:36–48. [PubMed: 27345696]

88. Assinger A, Kral JB, Yaiw KC, Schrottmaier WC, Kurzejamska E, Wang Y, Mohammad AA, Religa P, Rahbar A, Schabbauer G, Butler LM, Soderberg-Naucler C. Human cytomegalovirus-platelet interaction triggers toll-like receptor 2-dependent proinflammatory and proangiogenic responses. Arterioscler Thromb Vasc Biol. 2014; 34:801–809. [PubMed: 24558109]

89. Forghani B, Schmidt NJ. Association of herpes simplex virus with platelets of experimentally infected mice. Arch Virol. 1983; 76:269–274. [PubMed: 6307229]

90. Bik T, Sarov I, Livne A. Interaction between vaccinia virus and human blood platelets. Blood. 1982; 59:482–487. [PubMed: 7059666]

91. Youssefian T, Drouin A, Masse JM, Guichard J, Cramer EM. Host defense role of platelets: Engulfment of hiv and staphylococcus aureus occurs in a specific subcellular compartment and is enhanced by platelet activation. Blood. 2002; 99:4021–4029. [PubMed: 12010803]

92. Danon D, Jerushalmy Z, De Vries A. Incorporation of influenza virus in human blood platelets in vitro. Electron microscopical observation. Virology. 1959; 9:719–722. [PubMed: 13814035]

93. de Almeida AJ, Campos-de-Magalhaes M, Brandao-Mello CE, de Oliveira RV, do Espirito-Santo MP, Yoshida CF, Lampe E. Detection of hepatitis c virus in platelets: Evaluating its relationship to antiviral therapy outcome. Hepatogastroenterology. 2009; 56:429–436. [PubMed: 19579615]

94. Noisakran S, Gibbons RV, Songprakhon P, Jairungsri A, Ajariyakhajorn C, Nisalak A, Jarman RG, Malasit P, Chokephaibulkit K, Perng GC. Detection of dengue virus in platelets isolated from dengue patients. Southeast Asian J Trop Med Public Health. 2009; 40:253–262. [PubMed: 19323010]

95. Negrotto S, Jaquenod de Giusti C, Rivadeneyra L, Ure AE, Mena HA, Schattner M, Gomez RM. Platelets interact with coxsackieviruses b and have a critical role in the pathogenesis of virus-induced myocarditis. J Thromb Haemost. 2015; 13:271–282. [PubMed: 25393316]

96. Sottnek HM, Campbell WG Jr, Cassel WA. The pathogenesis of vaccinia virus toxicity. Ii. An electron microscopic study. Lab Invest. 1975; 33:522–532. [PubMed: 1186130]

97. Visser MR, Tracy PB, Vercellotti GM, Goodman JL, White JG, Jacob HS. Enhanced thrombin generation and platelet binding on herpes simplex virus-infected endothelium. Proc Natl Acad Sci U S A. 1988; 85:8227–8230. [PubMed: 2847155]

98. Onlamoon N, Noisakran S, Hsiao HM, Duncan A, Villinger F, Ansari AA, Perng GC. Dengue virus-induced hemorrhage in a nonhuman primate model. Blood. 2010; 115:1823–1834. [PubMed: 20042723]

99. Hottz ED, Lopes JF, Freitas C, Valls-de-Souza R, Oliveira MF, Bozza MT, Da Poian AT, Weyrich AS, Zimmerman GA, Bozza FA, Bozza PT. Platelets mediate increased endothelium permeability in dengue through nlrp3-inflammasome activation. Blood. 2013; 122:3405–3414. [PubMed: 24009231]

100. Rondina MT, Brewster B, Grissom CK, Zimmerman GA, Kastendieck DH, Harris ES, Weyrich AS. In vivo platelet activation in critically ill patients with primary 2009 influenza a(h1n1). Chest. 2012; 141:1490–1495. [PubMed: 22383669]

101. Singh MV, Davidson DC, Kiebala M, Maggirwar SB. Detection of circulating platelet-monocyte complexes in persons infected with human immunodeficiency virus type-1. J Virol Methods. 2012; 181:170–176. [PubMed: 22387340]

102. Passacquale G, Vamadevan P, Pereira L, Hamid C, Corrigall V, Ferro A. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. PLoS One. 2011; 6:e25595. [PubMed: 22022418]

103. Rondina MT, Schwertz H, Harris ES, Kraemer BF, Campbell RA, Mackman N, Grissom CK, Weyrich AS, Zimmerman GA. The septic milieu triggers expression of spliced tissue factor mrna in human platelets. J Thromb Haemost. 2011; 9:748–758. [PubMed: 21255247]

104. Rondina MT, Tatsumi K, Bastarache JA, Mackman N. Microvesicle tissue factor activity and interleukin-8 levels are associated with mortality in patients with influenza a/h1n1 infection. Crit Care Med. 2016; 44:e574–578. [PubMed: 26751613]

105. Boilard E, Pare G, Rousseau M, Cloutier N, Dubuc I, Levesque T, Borgeat P, Flamand L. Influenza virus h1n1 activates platelets through fcgammariia signaling and thrombin generation. Blood. 2014; 123:2854–2863. [PubMed: 24665136]
106. Le VB, Schneider JG, Boergeling Y, Berri F, Ducatez M, Guerin JL, Adrian I, Errazuriz-Cerda E, Frasquilho S, Antunes L, Lina B, Bordet JC, Jandrot-Perrus M, Ludwig S, Riteau B. Platelet activation and aggregation promote lung inflammation and influenza virus pathogenesis. Am J Respir Crit Care Med. 2015; 191:804–819. [PubMed: 25664391]
107. McMorran BJ, Marshall VM, de Graaf C, Drysdale KE, Shabbar M, Smyth GK, Corbin JE, Alexander WS, Foote SJ. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. Science. 2009; 323:797–800. [PubMed: 19197068]
108. McMorran BJ, Wieczorski L, Drysdale KE, Chan JA, Huang HM, Smith C, Mitiku C, Beeson JG, Burgio G, Foote SJ. Platelet factor 4 and duffy antigen required for platelet killing of plasmodium falciparum. Science. 2012; 338:1348–1351. [PubMed: 23224555]
109. Aggrey AA, Srivastava K, Ture S, Field DJ, Morrell CN. Platelet induction of the acute-phase response is protective in murine experimental cerebral malaria. J Immunol. 2013; 190:4685–4691. [PubMed: 23536632]
110. Grau GE, Mackenzie CD, Carr RA, Redard M, Pizzolato G, Allasia C, Cataldo C, Taylor TE, Molyneux ME. Platelet accumulation in brain microvessels in fatal pediatric cerebral malaria. J Infect Dis. 2003; 187:461–466. [PubMed: 12552430]
111. Risitano A, Beaulieu LM, Vitseva O, Freedman JE. Platelets and platelet-like particles mediate intercellular rna transfer. Blood. 2012; 119:6288–6295. [PubMed: 22596260]
112. Clancy L, Beaulieu LM, Tanriverdi K, Freedman JE. The role of rna uptake in platelet heterogeneity. Thromb Haemost. 2017; 117:948–961. [PubMed: 28276570]
113. Mause SF, von Hundelshausen P, Zernecke A, Koenen RR, Weber C. Platelet microparticles: A transcellular delivery system for rantes promoting monocyte recruitment on endothelium. Arterioscler Thromb Vasc Biol. 2005; 25:1512–1518. [PubMed: 15890969]
114. Mause SF, Ritzel E, Liehn EA, Hristov M, Bidzhekov K, Muller-Newen G, Soehnlein O, Weber C. Platelet microparticles enhance the vasoregenerative potential of angiogenic early outgrowth cells after vascular injury. Circulation. 2010; 122:495–506. [PubMed: 20644015]
115. Gidlof O, van der Brug M, Ohman J, Gilje P, Olde B, Wahlestedt C, Erlinge D. Platelets activated during myocardial infarction release functional mirna, which can be taken up by endothelial cells and regulate icam1 expression. Blood. 2013; 121:3908–3917. S3901–3926. [PubMed: 23493781]
116. Laffont B, Corduan A, Rousseau M, Duchez AC, Lee CH, Boilard E, Provost P. Platelet microparticles reprogram macrophage gene expression and function. Thromb Haemost. 2015; 115
117. Laffont B, Corduan A, Ple H, Duchez AC, Cloutier N, Boilard E, Provost P. Activated platelets can deliver mrna regulatory ago2*microrna complexes to endothelial cells via microparticles. Blood. 2013; 122:253–261. [PubMed: 23652806]
118. Liang H, Yan X, Pan Y, Wang Y, Wang N, Li L, Liu Y, Chen X, Zhang CY, Gu H, Zen K. Microrna-223 delivered by platelet-derived microvesicles promotes lung cancer cell invasion via targeting tumor suppressor epb41l3. Mol Cancer. 2015; 14:58. [PubMed: 25881295]
119. Michael JV, Wurtzel JGT, Mao GF, Rao AK, Kolpakov MA, Sabri A, Hoffman NE, Rajan S, Tomar D, Madesh M, Nieman MT, Yu J, Edelstein LC, Rowley JW, Weyrich AS, Goldfinger LE. Platelet microparticles infiltrating solid tumors transfer mirnas that suppress tumor growth. Blood. 2017
120. Jobe SM, Wilson KM, Leo L, Raimondi A, Molkentin JD, Lentz SR, Di Paola J. Critical role for the mitochondrial permeability transition pore and cyclophilin d in platelet activation and thrombosis. Blood. 2008; 111:1257–1265. [PubMed: 17989312]
121. Liu F, Gamez G, Myers DR, Clemmons W, Lam WA, Jobe SM. Mitochondrially mediated integrin alphaiibbeta3 protein inactivation limits thrombus growth. J Biol Chem. 2013; 288:30672–30681. [PubMed: 24014035]
122. McDonald B, Pittman K, Menezes GB, Hirota SA, Slaba I, Waterhouse CC, Beck PL, Muruve DA, Kubes P. Intravascular danger signals guide neutrophils to sites of sterile inflammation. Science. 2010; 330:362–366. [PubMed: 20947763]

123. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial damps cause inflammatory responses to injury. Nature. 2010; 464:104–107. [PubMed: 20203610]

124. Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM, Lima BH, Lopes GA, Russo RC, Avila TV, Melgaco JG, Oliveira AG, Pinto MA, Lima CX, De Paula AM, Cara DC, Leite MF, Teixeira MM, Menezes GB. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. Hepatology. 2012; 56:1971–1982. [PubMed: 22532075]

125. Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, Akira S, Yamamoto A, Komuro I, Otsu K. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. Nature. 2012; 485:251–255. [PubMed: 22535248]

126. Cloutier N, Tan S, Boudreau LH, Cramb C, Subbaiah R, Lahey L, Albert A, Shnayder R, Gobezie R, Nigrovic PA, Farndale RW, Robinson WH, Brisson A, Lee DM, Boilard E. The exposure of autoantigens by microparticles underlies the formation of potent inflammatory components: The microparticle-associated immune complexes. EMBO Mol Med. 2013; 5:235–249. [PubMed: 23165896]

127. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, Massarotti EM, Remold-O'Donnell E, Farndale RW, Ware J, Lee DM. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. Science. 2010; 327:580–583. [PubMed: 20110505]

128. Gyorgy B, Szabo TG, Turiak L, Wright M, Herczeg P, Ledeczi Z, Kittel A, Polgar A, Toth K, Derfalvi B, Zelenak G, Borocz I, Carr B, Nagy G, Vekey K, Gay S, Falus A, Buzas EI. Improved flow cytometric assessment reveals distinct microvesicle (cell-derived microparticle) signatures in joint diseases. PLoS One. 2012; 7:e49726. [PubMed: 23185418]

129. Mehta RS, Nishihara R, Cao Y, Song M, Mima K, Qian ZR, Nowak JA, Kosumi K, Hamada T, Masugi Y, Bullman S, Drew DA, Kostic AD, Fung TT, Garrett WS, Huttenhower C, Wu K, Meyerhardt JA, Zhang X, Willett WC, Giovannucci EL, Fuchs CS, Chan AT, Ogino S. Association of dietary patterns with risk of colorectal cancer subtypes classified by fusobacterium nucleatum in tumor tissue. JAMA Oncol. 2017; 3:921–927. [PubMed: 28125762]

130. Welsh JD, Muthard RW, Stalker TJ, Taliaferro JP, Diamond SL, Brass LF. A systems approach to hemostasis: 4. How hemostatic thrombi limit the loss of plasma-borne molecules from the microvasculature. Blood. 2016; 127:1598–1605. [PubMed: 26738537]

131. Leebeek FW, Boersma E, Cannon CP, van de Werf FJ, Simoons ML. Oral glycoprotein iib/iiia receptor inhibitors in patients with cardiovascular disease: Why were the results so unfavourable. Eur Heart J. 2002; 23:444–457. [PubMed: 11863347]

132. Leon C, Ravanat C, Freund M, Cazenave JP, Gachet C. Differential involvement of the p2y1 and p2y12 receptors in platelet procoagulant activity. Arterioscler Thromb Vasc Biol. 2003; 23:1941–1947. [PubMed: 12933533]

133. Sahni A, Sahni SK, Francis CW. Endothelial cell activation by il-1beta in the presence of fibrinogen requires alphavbeta3. Arterioscler Thromb Vasc Biol. 2005; 25:2222–2227. [PubMed: 16123330]

134. Zhang G, Han J, Welch EJ, Ye RD, Voyno-Yasenetskaya TA, Malik AB, Du X, Li Z. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via tlr4/myd88 and the cgmp-dependent protein kinase pathway. J Immunol. 2009; 182:7997–8004. [PubMed: 19494325]

135. Jenne CN, Wong CH, Zemp FJ, McDonald B, Rahman MM, Forsyth PA, McFadden G, Kubes P. Neutrophils recruited to sites of infection protect from virus challenge by releasing neutrophil extracellular traps. Cell Host Microbe. 2013; 13:169–180. [PubMed: 23414757]

136. Carestia A, Kaufman T, Rivadeneyra L, Landoni VI, Pozner RG, Negrotto S, D'Atri LP, Gomez RM, Schattner M. Mediators and molecular pathways involved in the regulation of neutrophil extracellular trap formation mediated by activated platelets. J Leukoc Biol. 2016; 99:153–162. [PubMed: 26320263]

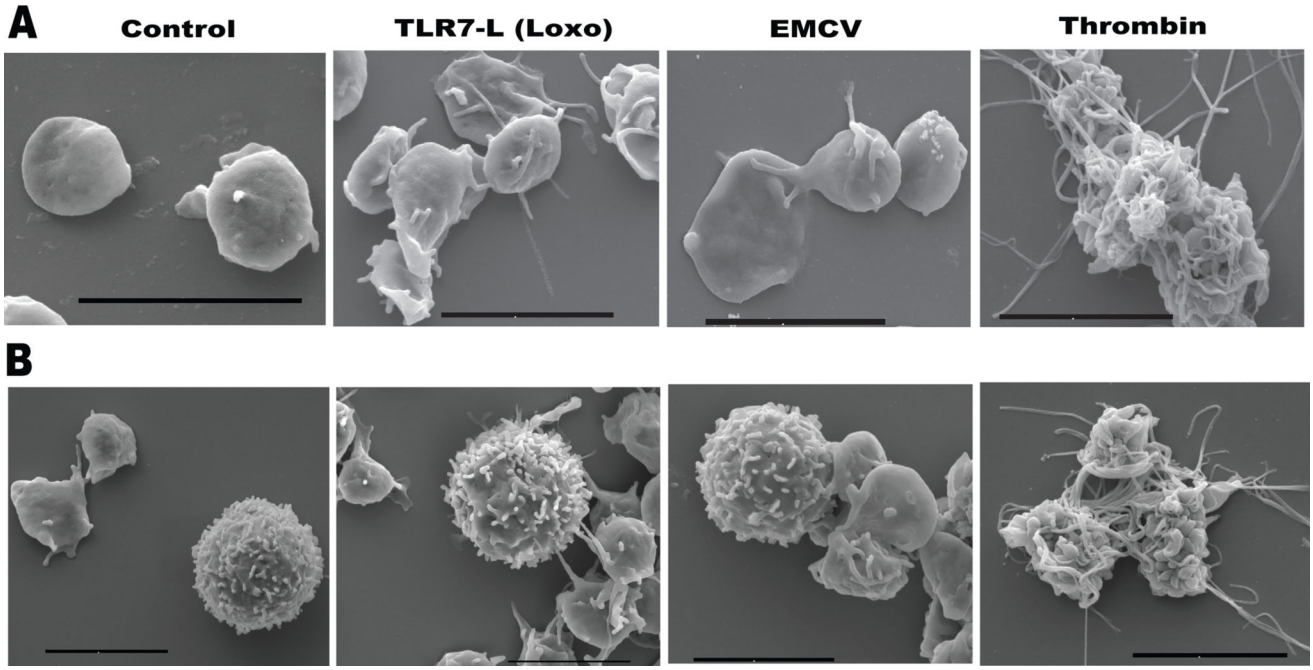

Figure 1. Differences in platelet thrombotic vs. immune activation visualized by scanning electron microscopy

Isolated **A.** human platelets or **B.** human platelets mixed with leukocytes, were treated with immune (Loxoribine-Loxo, Encephalomyocarditis virus-EMCV) or thrombotic agonists. Panels demonstrate differing levels of activation when platelets are activated via immune vs. thrombotic pathways.

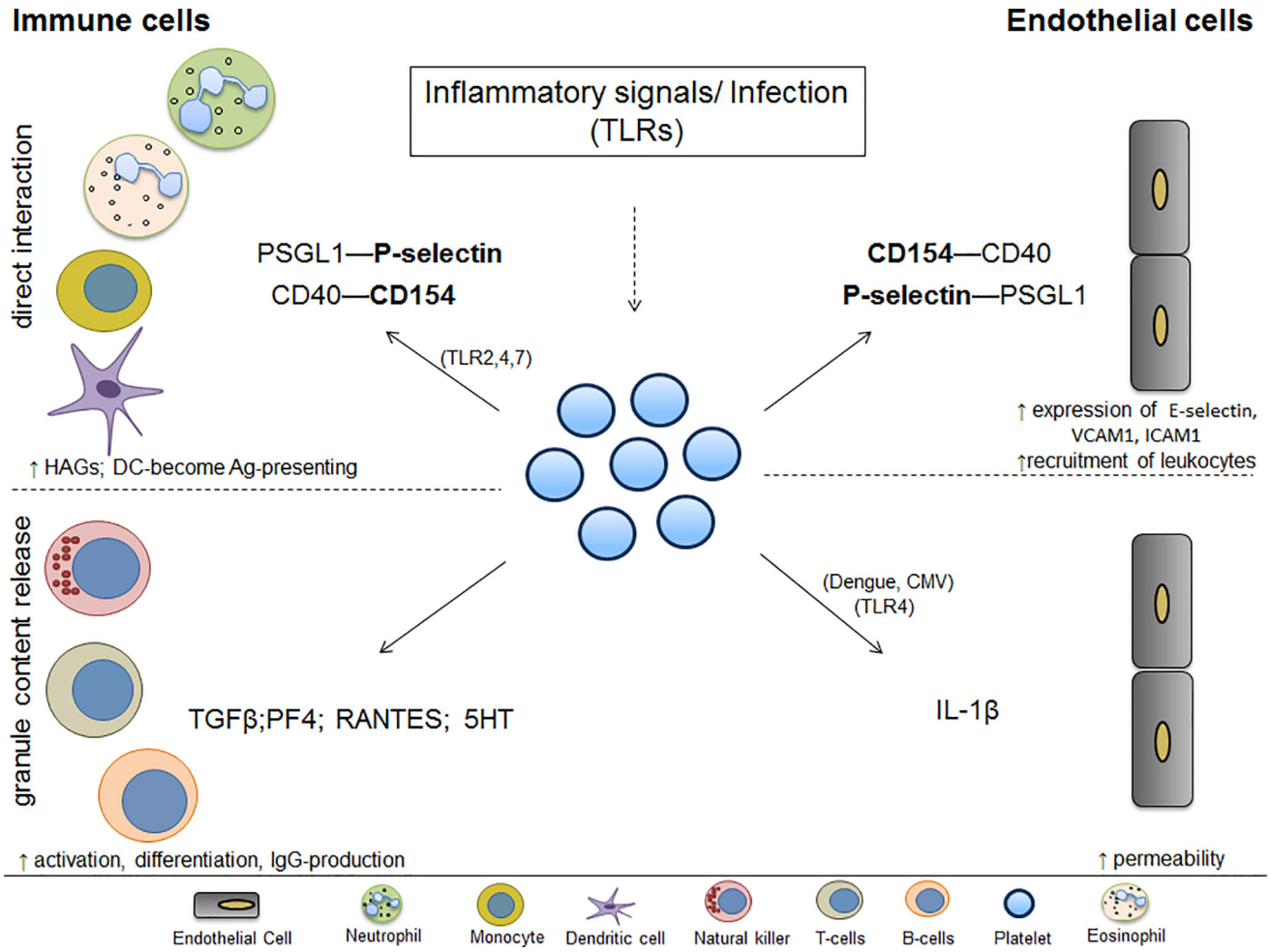

Figure 2. Platelet-mediated interactions with vascular or circulating cells

Platelets interact with endothelial and immune cells in the circulation, orchestrating a response to microbes, inflammatory stimuli and vessel damage. Through their TLRs (or inflammatory signals) platelets can change their surface expression and release their granule content thereby engaging different immune cells. Platelets form HAGs and initiate innate immune responses in the presence of TLR agonists and viruses such as EMCV, CVB, dengue, flu, HIV. Platelets can interact with DC through their P-selectin, activate them to become Ag-presenting through their CD154. By releasing α- or δ-granule content which leads to IgG (IgG1, IgG2, IgG3) production and control of T-cell function, platelets engage the adaptive immune response. Similarly, platelets are able to activate the endothelium, make it more permeable and mediate leukocyte trafficking to the inflamed endothelium. Proteins in bold represent changes of expression on the platelet surface. Continuous lines represent direct binding; dotted lines represent interaction through secretion. Abbreviations: HAG-heterotypic aggregates; Ag-antigen; 5-HT-serotonin; PF4-platelet factor 4; CMV-cytomegalovirus; EMCV-encephalomyocarditis virus; CVB-coxsackievirus B;

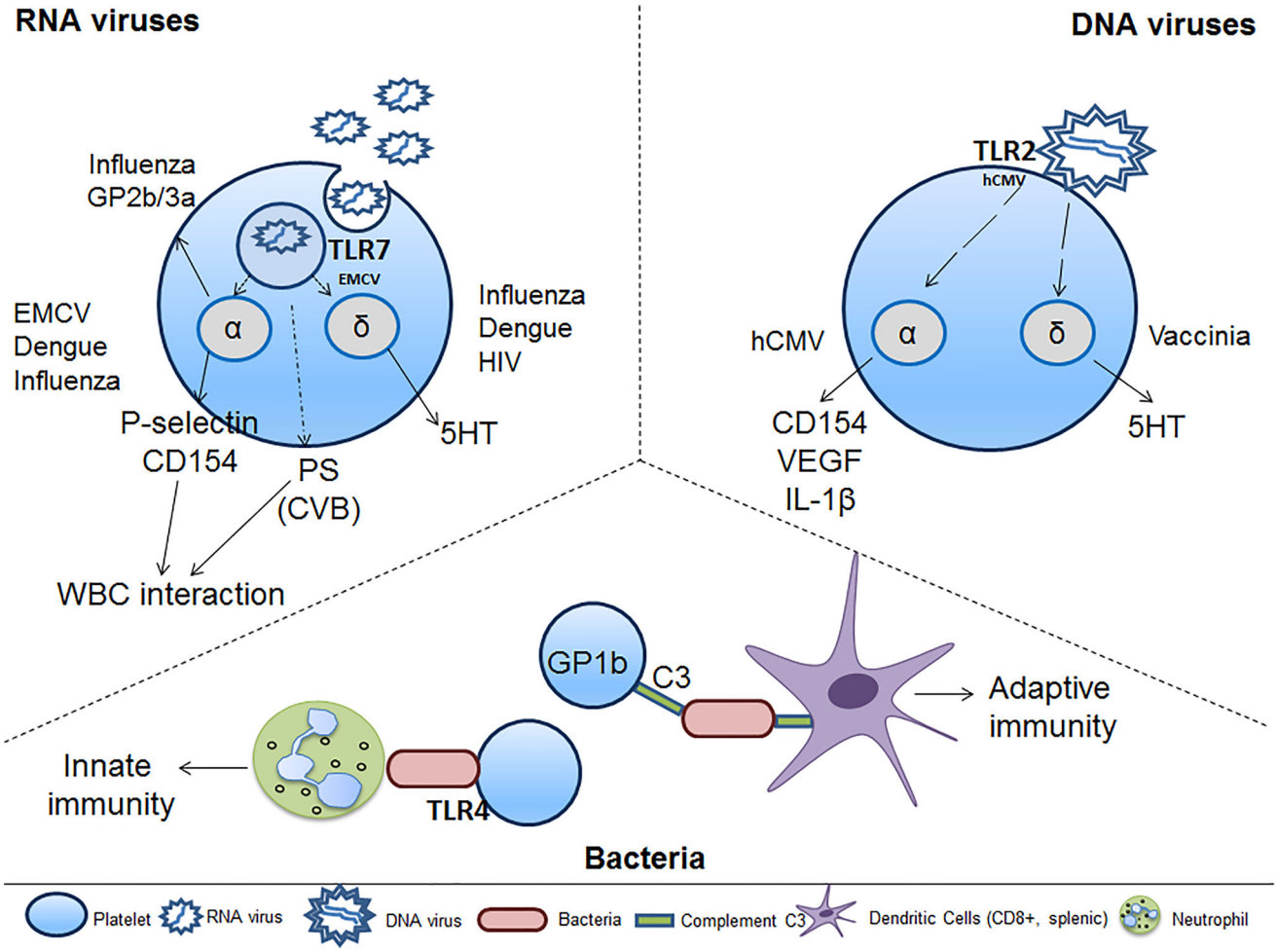

Figure 3. Platelet and circulating cell interactions during infection initiate the innate or adaptive immune response

Platelets achieve cell-to-cell communication during bacterial or viral infection either by direct interaction with WBCs through surface expression of platelet proteins or through indirect protein release from their α- or δ-granules. EMCV-activated platelets interact with neutrophils in a TLR7-dependent manner. CVB-activated platelets bind to neutrophils in a PS-dependent manner. Dengue and influenza increase microparticle release; dengue-mediated microparticles contain IL-1β. HCMV-activated platelets interact with neutrophils, monocytes, B-cells, T-cells and DCs, suggesting activation of innate and adaptive immune responses. Vaccinia-bound platelets have reduced aggregation potential in the presence of ADP, collagen, or thrombin. The specific pathways by which platelets respond to Herpes simplex virus (HSV)1 or HSV2 are currently unknown. During bacterial infection, platelet interactions with complement C3 opsonized bacteria through GP1b (CD42) lead to slowing of bacterial clearance. DCs recognize the platelet-bacterial complexes, thereby, inducing adaptive immunity. These platelet-bacterial interactions are true for Gram-positive or Gram-negative bacteria. Abbreviations: WBC-white blood cells; 5HT-serotononin; VEGF-vascular endothelial growth factor; PS-phosphatidylserine; C3-complement C3; EMCV-encephalomyocarditis virus; hCMV-human cytomegalovirus; CVB-coxsackievirus B; HIV-human immunodeficiency virus;

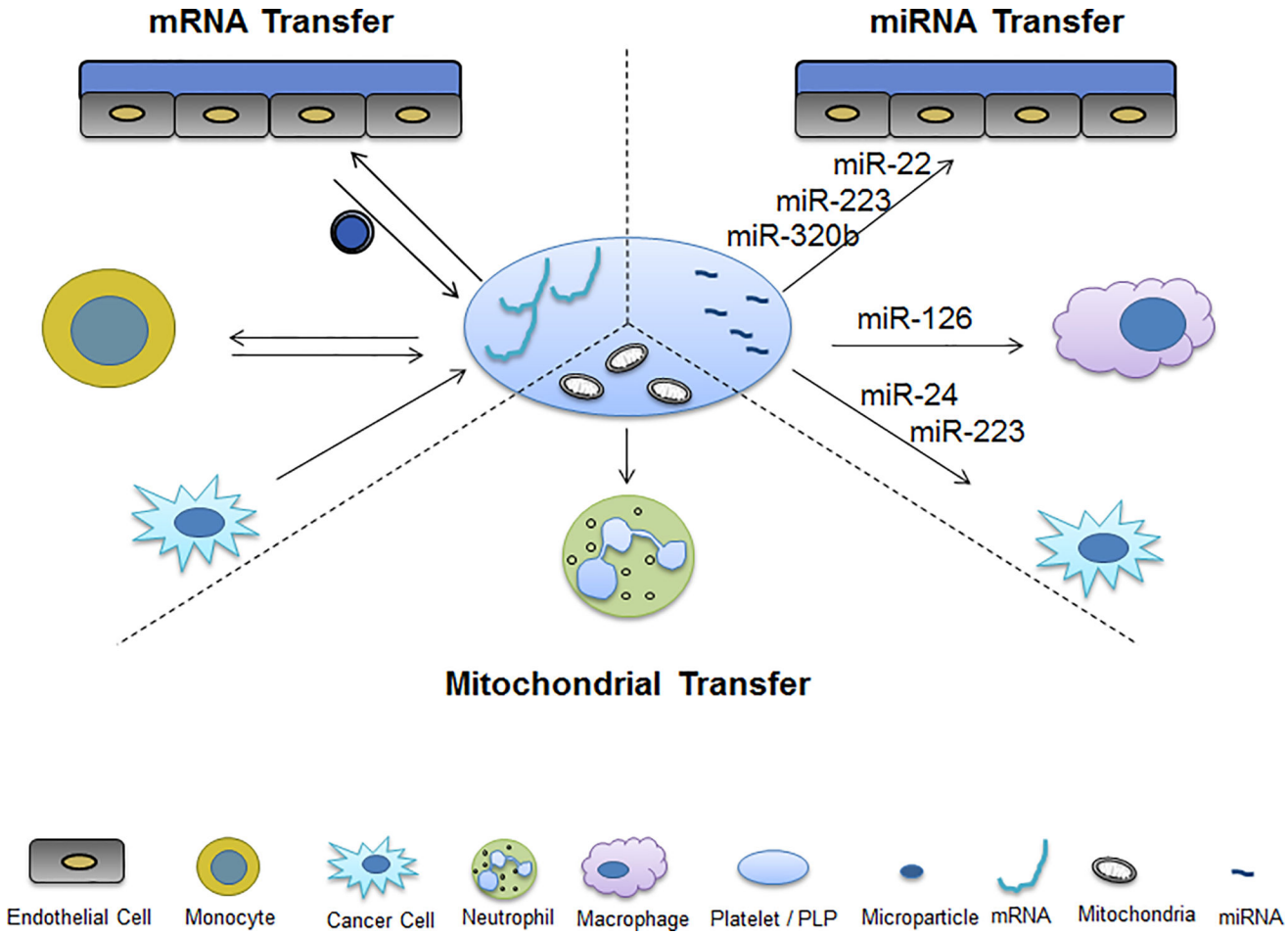

Figure 4. Platelet vascular and circulating cellular communication by non-protein-dependent mechanisms

Platelets and platelet-like particles (PLPs) have been shown to communicate with vascular cells in several protein-independent manners, specifically through mitochondrial and RNA transfer. RNA can be transferred from platelets to endothelial cells, monocytes, macrophages, and cancer cells. Inversely, RNA can be taken up by platelets from endothelial cells, monocytes, and cancer cells. Mitochondrial transfer from platelets to neutrophils can also occur. Microparticles mediate a significant number of these communication pathways.

Table 1

Platelet-derived mediators linking thrombosis, infection and immunity

Category of platelet mediator	Platelet receptor/ protein/ molecule	Vascular or /circulating cell interaction	Setting	Functions	Ref.
Integrins	GP1b (Cd42)	vWF; endothelial cells; leukocytes; bacteria	High shear stress / infection	unfolded vWF deposited on collagen; endothelial P-selectin; binds thrombin limiting leukocyte recruitment; binds complement C3 on bacteria to enhance adaptive immunity.	15, 18, 87
	GP1a/2a	subendothelial collagen	low shear stress	collagen	8
	GP2b/3a (CD41)	other platelets through Fgn; T-cells; bacteria	hemostasis; infection; immunity	platelet aggregation; endothelial ICAM1 or αVβ3 leading to firm adhesion; aggregation with activated T-cytolytic and T-helper cells; aggregation around bacteria; increase after H1N1 infection	8, 57,81, 105
α-granule proteins	PF4 (CXCL4)	T-cells; monocytes; RBCs; bacteria	infection; atherosclerosis	limit Th17 expansion and differentiation; monocyte recruitment (heterodimer with RANTES); inhibits TGFβ signaling; binds gram-negative bacteria increasing opsonization; kill plasmodium in RBCs	60, 61, 83, 84, 107–109
	RANTES (CCL5)	immunity; atherogenesis	infection; chronic inflammation	T-cell activation and differentiation; monocyte/macrophage adhesion and recruitment;	29, 53, 60
	TGFβ	tumor cells (TGFβR)	metastasis	reduce NK antitumor activity; contribute to induction of invasive epithelial mesenchymal transition to metastasis	65, 72, 73
	β-defensin	neutrophils	*S. aureus* alpha toxin	netosis	48
δ-granule molecules	Serotonin (5HT)	endothelial cells; T-cells	hemostasis/thrombosis; adaptive immunity	constricts injured blood vessels; enhances platelet aggregation to minimize blood loss; T-cell activation and differentiation; endothelial cell proliferation	27, 28
	ADP	platelet P2Y12; P2Y1	hemostasis	platelet recruitment, activation and aggregation during clot formation; exposure of P-selectin (P2Y12, P2Y1)and PS and thrombin generation (P2Y12)	132
surface protein expression	P-selectin	leukocyte PSGL1 (neutrophils, monocytes, DC) endothelial PSGL1 metastatic cells PSGL1	infection; other platelets	platelet-neutrophil and platelet-monocyte HAGs; interactions of leukocytes with the thrombi; platelet-DC interactions; increase as a result of TLR7 stimulation; metastatic PSGL1 adhesion	5, 6, 15, 62, 71
	PSGL1	endothelial P-selectin	high shear stress	platelet-endothelial interactions for thrombus formation in small venules	18

Category of platelet mediator	Platelet receptor/ protein/ molecule	Vascular or /circulating cell interaction	Setting	Functions	Ref.
	CD40	leukocyte CD154	inflammation/ Infection/ immunity	Surface expression as a result of TLR7platelet-neutrophil tethering to the endothelium; platelet-DC leading to T-cell antigen presentation	5, 40
	CD154	endothelial CD40	inflammation/ infection	increase in endothelial expression of E-selectin, VCAM1 and ICAM1, as well as secretion of MCP1 and IL-8	21
synthesized/ secreted	IL-1β	endothelial IL-1R associated with αVβ3 in the presence of Fgn	infection; inflammation	increases endothelial permeability by secreting NO	133
	TxA2	thromboxane A2 Receptor	hemostasis;	platelet recruitment, activation and aggregation during clot formation	5

* abbreviations: Fgn-fibrinogen, vWF-von Willebrand factor; RBC-red blood cell; NK-natural killer cell; PS-phosphatidylserine; PSGL1-P-selectin glycoprotein ligand 1; DC-dendritic cells; NO-nitric oxide; TxA2-thromboxane A2; HAG- heterotypic aggregates;

Circ Res. Author manuscript; available in PMC 2019 January 19.

Table 2

Platelet TLRs and their contribution to thrombosis and immunity

TLRs	Agonist	Interaction with cells	Result	Thrombosis	Platelet TLR-contribution to netosis	Organ netosis	Ref.
TLR2	Pam3CK4; $oxPC_{CD36}$	neutrophils	Platelet-neutrophil HAGs through P-selectin; activation of GP2b/3a hyperreactivity and the prothrombotic state in the setting of hyperlipidemia	+	+ 136	?	35, 36
TLR4	LPS	neutrophils, monocytes	Platelets reduce the time of netosis *in vivo*; platelets aggregate in TLR4/MyD88- and cGMP/PKG-dependent pathway	+	+	+	33, 134
TLR3	pIC; pAU		Activation of NF-kappaB, PI3K-Akt and ERK1/2 pathways in platelets; at high agonist concentrations may potentiate platelet aggregation; liver netosis (pIC)	—	?	+	41, 42
TLR7	Loxoribine; R838	neutrophils	Modest increase of P-selectin and CD40 through AKT and p38-MAPK; no effect on platelet aggregation; liver netosis (R838)	—	?	+	40, 135
TLR9	CpG; Carboxy-alkylpyrrole protein adducts (CAPA)		Platelet clumping in presence of collagen (CpG); platelet activation, granule secretion, and aggregation *in vitro* and thrombosis *in vivo* via the TLR9/MyD88 pathway (CAPA)	—	?	?	38, 39

* TLR3 may potentiate platelet aggregation at very high concentrations (50 μg/μl); Mitochondrial DNA can mediate netosis through TLR9.

Abbreviations: HAG- heterotypic aggregates; LPS-lipopolysaccharides; pIC- poly(I:C); pAU –poly(A:U);