



Inflammatory vs. Thrombotic States of Platelets: A Mini-review with a Focus on Functional Dichotomy

Seki R and Nishizawa K*

School of Medical Technology, Teikyo University, Japan

Abstract

In addition to roles in hemostasis and thrombosis, the role of platelets in inflammation and immunity have been the focus of recent studies. Both proinflammatory and prothrombotic roles of platelets are considered important in the pathophysiology of sepsis as well as in the protection from severe infections. The facilitation of prothrombotic activity by the proinflammatory activity of platelets has been shown in many studies. Nonetheless, some studies have highlighted the dichotomy of platelet functionality. Within a limited time period and space, only proinflammatory or prothrombotic functionality is often present. In some cases, the activation of the proinflammatory function of platelets is not accompanied by prothrombotic activity. Recent analyses of pattern recognition receptors such as toll-like receptors (TLRs) in platelets motivate us to consider how pathways are activated in innate immunity compared with pathways for thrombogenesis and hemostasis. In this article, we mainly discuss recent studies showing the functional dichotomy of platelets and, in particular, the conditions and possible mechanisms that activate platelets in a biased manner, rather than the concordance between prothrombotic and proinflammatory pathways in platelets.

Platelets in inflammation and thrombosis

Platelets play a central role as essential effectors in thrombosis, hemostasis, and inflammation [1,2]. A growing body of recent studies have revealed the capability of platelets to coordinate inflammation, immune, prothrombotic responses, angiogenesis, and wound healing [3]. In this section, we mainly discuss findings favoring the role of platelets in inflammation and innate immunity closely coupled with those for thrombosis and coagulation. Of note, we do not cover important adhesion receptors in hemostasis such as GP VI (glycoprotein VI) interactions with collagen [4] as well as GP IIb/IIIa (integrin α IIB β 3) [5]. We also refer readers to Yun et al. [6] and Cimmino [7] for introductory articles on the platelet biology focusing on activators, as well as Li et al., Thomas et al., and Smyth et al. for articles with a broader scope [1,2,4].

In sepsis, inflammation and coagulation are considered coupled to each other. Inflammation leads to a procoagulant state, and coagulation augments inflammation. The risk of acute thrombotic events increases during bacterial infections or inflammation [8]. A number of molecules and systems have been shown to be involved in reciprocal interrelationship between coagulation and innate immune systems [9]. An example for the linkage from inflammation to coagulation is tissue factor (TF) expression on the cell surface of leukocytes, induced by stimulation with endotoxin, tumor-necrosis factor (TNF)- α , or CD40 ligand (CD40L) [10]. TF is a receptor and cofactor for factor VII and VIIa and the key initiator of coagulation. TF is expressed on subendothelial mural cells and fibroblast around the vessel wall, and activated endothelial cells as well; however, the contribution of microvesicle-mediated transfer from monocytes is difficult to

quantify *in vivo* [11]. As another example, histamine, TNF- α , IL-8, and IL-6 all induce the release of large von Willebrand factor (vWF) multimers from the endothelium [12]. These large multimers potently promote platelet thrombi formation [12]. Furthermore, as the term immunothrombosis implies, both immune cells and thrombosis-related molecules act in a concerted manner to generate an intravascular scaffold to facilitate the containment and destruction of pathogens [13]. Thrombin plays an important role in protective immunity as shown by a mouse model for *Klebsiella pneumoniae* infections [14]. The authors also showed that the thrombin effects are dependent on platelet-neutrophil interactions and platelet protease-activated receptors (PAR)1 *in vivo*.

Given such interplay between the systems, it seems reasonable to assume that activated platelets should always show both proinflammatory and prothrombotic properties (which we refer to as 'concordance in platelet activation'). Platelets are important in sepsis pathophysiology where both inflammation and coagulation are central issues [e.g., 15]. Activated platelets release a variety of prothrombotic and proinflammatory mediators, and recruit leukocytes including monocytes, neutrophils, and lymphocytes to sites of vascular injury and inflammation [1]. Detailed analyses of platelets further validate the view of the general concordance of proinflammatory and prothrombotic platelet activation. For instance, α -granules contain not only P-selectin and chemokines including platelet factor 4 (PF4, CXCL4), macrophage inflammatory protein (MIP)-1 α and RANTES that jointly help recruit/activate leukocytes, but also platelet endothelial adhesion molecule-1, GPIIb/IIIa, and vWF, which are the key players in thrombosis and hemostasis [2]. It is also known that inflammatory mediators, such as IL-6, increase platelet production, and the newly formed platelets are likely

to be more thrombogenic [16]. Components of the activated complement system can confer platelets with increased procoagulant activity, likely contributing to thrombotic events [17-19].

The concordance between proinflammatory and prothrombotic activation of platelets can also be supported by the presence of the molecules with such dual roles in the platelets. For example, the binding of P-selectin of platelets with P-selectin glycoprotein ligand-1 (PSGL-1) expressed on leukocytes is necessary for leukocyte trafficking and translocation, but PSGL-1 is also carried by monocyte-derived microparticles as well, and P-selectin/PSGL-1 interaction is also essential for growth of the thrombus at sites of injury [17,20]. CD40L, in membrane-bound and soluble forms, exerts diverse proinflammatory and prothrombotic effects by binding mainly to CD40 on the surface of vascular walls and circulating cells [9]. Another example is polyphosphate, which is contained in platelet dense granules [21]. Polyphosphate initiates factor XII-driven fibrin formation (the intrinsic pathway of coagulation) on the surface of activated platelets. Furthermore, active factor XII causes the generation of active kallikreins, which leads to the liberation of bradykinin, a well-known inflammatory mediator, from high-molecular-weight kininogen [22].

Despite such findings in support of inseparable coupling of the immune/inflammatory and the prothrombotic platelet activation, when we focus on the activities of platelets within a limited spatial or temporal window, only one of the proinflammatory and prothrombotic activities is found to be activated in certain settings. Moreover, the multiplicity of platelet functions in angiogenesis even appears to have conflicting functionalities; PAR1, ADP (P2Y₁ and P2Y₁₂), and GP VI stimulation promotes proangiogenic effects (releasing stromal derived factor [SDF]-1 α , CXCL12, and vascular endothelial growth factor [VEGF]), whereas PAR4 promotes anti-angiogenic effects such as the release of PF4 and endostatin [3,23,24]. However, it remains largely unknown what differences in activated signaling pathways lead to the activation of selected functionalities.

Question 1: What is dichotomy in platelet activation?

As we have discussed above, the activation of proinflammatory and immune functionalities of platelets appears to be accompanied by their prothrombotic functionalities. However, only one of the proinflammatory and prothrombotic activities has been shown to be activated in some settings. To our knowledge, such a view, namely, platelet dichotomy, dates back to an article by Page [25]. It was unclear why platelet activation by inflammatory mediators did not result in thrombosis and coagulation *in vivo* [25,26]. Later, Page, Pitchford and coworkers investigated why increased risk of thrombosis was not observed in patients with asthma and allergic animal models, despite the clear occurrence of platelet activation and P-selectin mediated leukocyte recruitment, a pivotal role for platelets in inflammation [27]. Rather than increased thrombosis, mild hemostatic defects have been reported to be associated with asthma [28].

Here, a brief introduction on purine receptors and signaling pathways may help. Contained in dense granules, ADP serves as a prominent amplifier of initial platelet activation and as a cofactor to all physiological platelet activators including thromboxane A₂, collagen, and thrombin [29]. ADP-induced platelet activation is initiated by P2Y₁ receptor and amplified by P2Y₁₂ receptor [30]. Compared to P2Y₁ that mediates quick response and transient platelet aggregation, P2Y₁₂ potentiates platelet secretion and sustained aggregation [31]. Thus, in the context of thrombosis *in vivo*, both P2Y₁ and P2Y₁₂ receptors are important [32]. *In vitro* systems using platelet-rich plasma (PRP) that lack leukocytes, P2Y₁₂, but not P2Y₁ are involved in thrombin generation and phosphatidylserine (PS) exposure, which provides a catalytic surface for enzyme reactions required for coagulation. *In vitro* analyses by Leon et al. suggested that the P2Y₁₂-G_i-PI3 kinase (PI3K) pathway, compared with P2Y₁, has a more important role in the exposure of procoagulant catalytic surfaces. Their whole blood/ADP (or thrombin receptor activating peptide [TRAP]) stimulation systems with both P2Y₁ and P2Y₁₂ receptors are important for platelet P-selectin expression and TF-expression of leukocytes. Thus, P2Y₁₂ appeared essential in all experiments on thrombogenesis and PS exposure [32], and is also involved in P-selectin expression and platelet-leukocyte complex formation. In terms of signaling pathways, such as PAR receptors, these P2Y receptors belong to GPCR receptors. It should be noted that, besides GPCRs, platelets express the receptors/integrins that directly or indirectly activate Src family kinases, TLRs, and receptor of other types [4].

Strikingly, in the context of allergic inflammation, P2Y₁₂ does not to play a significant role. Specifically Amison et al. used an antigen ovalbumin (OVA) challenge for a IgE-dependent lung inflammation mouse model. In their study, analysis of the antagonists revealed that P2Y₁ but not P2Y₁₂ stimulation leads to formation of circulating platelet-leukocyte complexes in a manner dependent on allergen exposure [26,33]. Thus, in their inflammation model, P2Y₁ activation was necessary for the formation of the platelet-leukocyte complex, but P2Y₁₂ activation was not involved in this process. On the other hand, ADP-P2Y₁ alone did not lead to platelet-platelet aggregation in their system, consistent with the well-known importance of P2Y₁₂ in thrombotic events.

Later, the authors also showed that antagonists of P2Y₁ and P2Y₁₄, but not of P2Y₁₂, inhibited the intranasal lipopolysaccharide (LPS) administration-induced pulmonary neutrophil recruitment [34]. Here, the P2Y₁ antagonist, but not the P2Y₁₂ antagonist, suppressed platelet-dependent neutrophil motility toward macrophage-derived chemokine (MDC), whose receptor (CCR4) is expressed on platelets but not on neutrophils. On the other hand, P2Y₁ and P2Y₁₂ were equally important for ADP-induced platelet aggregation *ex vivo*. These findings corroborate those of Liverani et al. [35], who argued against the idea that P2Y₁₂ is important for inflammation; they showed that the levels of inflammation (numbers of white blood cells, plasma levels of cytokines, and the severity of lung injury) in LPS-treated P2Y₁₂-knockout mice were higher than those in LPS-treated wild-type mice. It should be noted, however, that P2Y₁₂ is likely to be expressed

in not only platelets but also in lymphocytes, monocytes, and dendritic cells [36,37]. In contrast, P2Y₁₂ is required for the development of leukotriene E4-induced pulmonary inflammation [38]. Nonetheless, the use of anti-P2Y₁₂ drugs in the studies by Amison et al. [34] and Liverani et al. [35] thus showed that distinct functionalities were associated with distinct sets of receptor/signaling pathways.

Amison et al. demonstrated that P2Y₁₂ was not important for inflammation by showing that the platelet-leukocyte interaction is mainly mediated by the P2Y₁-RhoA pathway [26]. This coincides with the finding from Pleines et al. that the P2Y₁-RhoA axis is important for platelet-leukocyte complexes and leukocyte chemotaxis [39]. RhoA, in other cells, is known to be important for actin cytoskeleton remodeling, cell polarity and chemotaxis [40].

Here, we consider the possibility that switching the active/inactive states of RhoA plays a pivotal role in switching the platelet between the proinflammatory and prothrombotic states. Of note, G₁₃ and G_q are upstream of the RhoA pathway [39]. The role for G₁₃-RhoA-rho-associated coiled-coil containing protein kinase (G₁₃-RhoA-ROCK) pathway and G_q-induced myosin light chain (MLC) phosphorylation in platelet shape change have been reported as discussed in Pleines et al. [39]. Furthermore, α-granule release is likely mediated by both the G₁₃-RhoA and G_q-RhoA pathway [39,41]. Contrary to G₁₃- and G_q-coupled receptors, it is likely that P2Y₁₂, which is coupled to G_i, is not tightly involved in the RhoA pathway [4] (Figure 1).

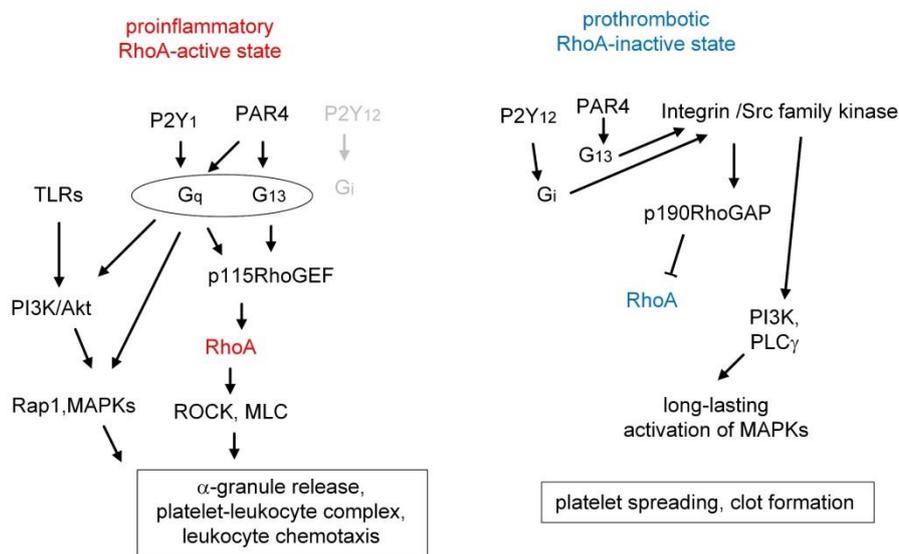


Figure 1: Alternative states of signaling pathway activation of platelets, based on our hypothesis of RhoA state associated with the proinflammatory and prothrombotic states. p115RhoGEF, p115 rho guanine nucleotide exchange factor; p190RhoGAP, p190 rho GTPase-activating protein, ROCK, rho-associated coiled-coil containing protein kinase; MLC, myosin light chain phosphorylation.

Additionally, p190RhoGAP is a negative regulator of RhoA. Bartolomé, et al. showed that the constitutively active mutant of G_{α13} leads to inhibition of RhoA activation, and this is mediated by p190RhoGAP [42,43]. It is possible that this inhibition takes place at the later stages in the thrombotic process. Therefore, we hypothesize that, during the early phase of thrombosis, as in the case of inflammation, thrombin-PAR4-G_{α13}-p115RhoGEF-RhoA axis as well as the ADP-P2Y₁₂-G_q-p115RhoGEF-RhoA axis is activated, leading to ROCK activation and MLC phosphorylation, thereby promoting platelet shape change and secretion [4,44]. After this initial stage during the course of prothrombotic activation, integrin-dependent inhibition of RhoA may take place in platelets in the following manner. That is, G_{α13}-GPCR-integrin crosstalk begins, in which the cytosolic domain of integrin β3-G_{α13} interacts [45]. This interaction causes Src family kinase (SFK) activation and thus phosphorylation (activation)

of p190RhoGAP, which inhibits RhoA [43,45-47]. Of note, thrombosis proceeds even in the RhoA inactive phase; the inhibition of RhoA does not affect thrombosis, as platelet aggregation was not affected in RhoA-deficient mice [39]. As it is well-known that integrin β3 (of αIIbβ3 = GPIIb/IIIa) plays a central role in the final process of thrombosis, we hypothesize that the active phase of p190RhoGAP corresponds to the phase in which GPIIb/IIIa (through conformational changes) can bind to fibrinogen in stabilizing platelet aggregation [5].

To summarize, we hypothesize that RhoA activity is important for inflammation and the early activation phase of thrombosis, but not for the phase that follows during thrombosis. Importantly, Gong et al. [45] proposed a discrete spatiotemporal regulation of RhoA in platelets, in which RhoA activation during the initial phase of platelet activation, its inhibition during the spreading process, and its reactivation

in the late phase of activation during clot retraction are proposed to occur. We hypothesize that the proinflammatory state of platelets may have some similarity to the early phase of prothrombotic change of platelets. However, further studies on the activate/inactive states of RhoA are necessary to address the dichotomous behavior of platelets.

Question 2: How do platelet activations through TLRs compare with classical pathways?

As the first line of defense, platelets detect pathogenic components through receptors expressed on the surface, and secrete or express a variety of cytokines and molecules that modulate activity of vascular and immune cells including leukocytes. Platelets express pattern recognition receptors (PRR) of different classes, including those from C-type lectin receptors and toll-like receptor (TLR) families [48,49]. Platelets have been shown to be expressed in virtually all TLRs (1–10 in human) [48,49]. For platelets, the functionalities of TLR2 and TLR4 have been relatively well characterized [50]. In this section, we consider in a non-systematic manner a few studies on TLR2, TLR4, and endosomal TLRs (i.e., TLR3, TLR7, and TLR9) [51,52], with an focus on TLR signaling in comparison with classical prothrombotic pathways. TLR3, TLR7, and TLR9 (endosomal TLRs) in platelets have been well studied. For TLR3, stimulation of isolated platelets with agonists has been shown to translocate α -granule-stored factors (P-selectin and CD40L) to the surface and induce enhanced procoagulant responses to traditional agonists including thrombin [53].

TLR2

TLR2 recognizes lipoproteins of gram-positive bacteria and functions in combination with TLR1 and TLR6. Blair et al. showed that TLR2 stimulation elicits a wide range of activation for both prothrombotic and proinflammatory responses [54]. Adhesion to the collagen matrix, P-selectin expression, and PF-4 secretion were all triggered by Pam3CSK4 treatment. With respect to their dichotomous nature, we note that these experiments were shown in independent settings, and not in one experiment. Interestingly, in whole blood stimulation, the formation of platelet-neutrophil aggregates was enhanced by Pam3CSK4, but platelet aggregation was not [54]. This implies that Pam3CSK4 (TLR2/1 agonist) treatment activates or prime platelets, but does not cause specification toward the proinflammatory state. Rather, TLR2 stimulation also potentiates platelets to respond to prothrombotic environments as well. Blair et al. further showed that TLR2 stimulation activates the PI3K/Akt pathway. This is not preceded by integrin activation because blockade of fibrinogen binding to GP IIb/IIIa (integrin α IIb β 3) interaction did not affect PI3K activation. PI3K has been shown to play a role in shape change, secretion, and inside-out activation, and therefore is considered important for both proinflammatory and prothrombotic activation of platelets. The PI3K/Akt pathway is likely to be a key pathway downstream of TLRs necessary for many functional responses of platelet.

Of importance, intracellular signaling induced by thrombin in comparison with those induced by the TLR2 ligand showed a kinetic difference that may serve as a basis for the biased activation of prothrombotic or proinflammatory properties of platelets [55]. Thrombin stimulation of PAR1/4 is known to activate both PI3K/Akt and PLC β pathways [4]. Akt and p38 phosphorylation was slower with Pam3CSK4 treatment than with thrombin treatment [55]. Moreover, Akt, p38, and ERK1/2 phosphorylation was only temporary after Pam3CSK4 treatment while it was stable with thrombin treatment. This slow and temporary effect of the TLR2 ligand may reflect the relatively slow feature of the inflammatory process. This is unsurprising considering that PAR1/4 is mainly coupled to G_q, and the G_q-PLC-IP₃-Ca²⁺ pathway has been well characterized in cell physiology as a rapid pathway; for instance, in the case of G_{q/11} coupled receptor-PLC-IP₃-Ca²⁺ pathway, following serum stimulation of fibroblasts, the Ca²⁺ level immediately peaks within 1 s of stimulation [56]. PI3K inhibitor sensitivity was clear for Pam3CSK4 stimulation but not for thrombin stimulation, suggesting the dependence on the PI3K pathway is clearer for TLR2 signaling but not for PAR1/4 signaling. Importantly, thrombin but not Pam3CSK4 induced platelet aggregation in their fibrin clot-retraction assay [55]. These studies suggest that PAR1/4 stimulation leads to a pronounced and wider range of pathway activation compared to TLR2 stimulation. By contrast, in whole blood, stimulation with Pam3CSK4 but not thrombin caused an increase in the number of platelet-monocyte complex [55].

It would be reasonable to envisage that the primary role of platelet TLR2 is to assist neutrophils protecting against bacterial infection. In periodontitis, the number of the platelet-leukocyte complex is increased [57]. Intriguingly, neutrophil-mediated phagocytosis of periodontopathogens (*Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*) was enhanced by platelets, although full enhancement required opsonization by complement [58]. The presence of platelets facilitated the attachment of the bacteria to neutrophils, and this effect required TLR2 on the platelets, although blocking analyses showed a synergistic effect of TLR4. Platelets also assisted neutrophil activations, on the basis of increased CD11b and decreased CD62L levels on the neutrophils. This finding adds to the view that platelets assist neutrophils in protecting against bacterial infection.

It is important to note that TLR2 signaling appears suitable for the platelet-neutrophil complex, yet does not appear to be a strong activator in a conventional sense. Of note, in a study by Ward et al., the TLR4 agonist LPS and TLR2 agonist Pam3CSK4 showed no direct effect on platelet activation [50]. Thrombin receptor activating peptide (TRAP)-6 induced pronounced CD63 expression (platelet activation marker), but only a modest level of platelet-neutrophil complex formation, compared with TLR2L. Indeed, Assinger et al. [58] demonstrated that TLR2/PI3K/Akt pathway activation was necessary in platelet-leukocyte interactions, but TRAP-6 stimulation appears to be dependent on PLC but not on PI3K/Akt (based on CD63 expression). This finding corroborates that of Rex [55], who

showed a dependence of Pam3CSK4-induced α -granule release on the PI3K/Akt signaling, whereas thrombin-induced α -granule release was not affected by the PI3K/Akt inhibitor as we mentioned above. However, further studies are necessary given the complex feature of PAR1/4 signaling; in fact, PAR1/4-induced Akt activation shows a biphasic nature, as well as phase-dependent involvement of PLC and PI3K [59],

Using human blood, Keane et al. showed that *Streptococcus pneumoniae* IO1196 induces platelet aggregation in a TLR2-dependent manner [60]. It was also shown that *S. pneumoniae* IO1196 can bind to HEK293 cells stably transfected with TLR2 but not with TLR4. Inhibition of TLR2 but not TLR4 completely abolished *S. pneumoniae*-induced platelet aggregation. Keane et al. further reported PI3K/RAP1 activation following TLR2 stimulation.

Intriguingly, it was shown that Pam3CSK4 (TLR2/1 agonist), but not MALP-2 (TLR2/6 agonist), induces cytoplasmic Ca^{2+} increase, TxB_2 production, dense granule secretion, and platelet aggregation [61]. However, these are indirect effects of TLR2 agonist; Pam3CSK4-induced platelet aggregation and secretion depends on P2X₁-mediated Ca^{2+} mobilization and ADP receptor activation. To demonstrate the slowness of TLRs signaling, Pam3CSK4 did not stimulate a rapid (within 10 minutes) phosphorylation of IRAK-1 in platelets.

TLR4

Data regarding the effect of TLR4 activation on platelets are somewhat controversial [50,62], although we do not discuss in detail here. It may be that the effect of TLR4 stimulation is not as strong as TLR2 stimulation. For example, the TLR4 ligand LPS was shown to have weaker activating effects relative to the TLR2 ligand Pam3CSK4, or just priming effects that assist thrombin stimulation [63]. In support of this, Pires et al. showed that LPS potentiates platelet responses via TLR4-stimulated Akt-ERK-PLA₂ signaling. Here, LPS did not significantly induce isolated human platelet aggregation, although LPS enhanced the aggregation of platelets stimulated by thromboxane (U46619) or GP VI collagen receptor agonists (CRP-XL) [63].

Analyses using anti-platelet agents also show controversial results. In Thomas [2,64], which was based on LPS injection experiments in human healthy volunteers, the P2Y₁₂ antagonist ticagrelor significantly reduced the formation of the platelet-monocyte complex and also reduced levels of inflammatory cytokines and D-dimer. Ticagrelor also suppressed prothrombotic changes in the fibrin clot ultrastructure. Furthermore, LPS administration did not cause detectable platelet aggregation, which was likely because of the weak effects of TLR4 stimulation. Nonetheless, the platelet inhibitor in this model has clinical importance. Similar effects of ticagrelor was reported by another laboratory [65]. However, given the disappointing results with prasugrel in the study by Schoergenhofer et al. [66], we cannot rule out the possibility that ticagrelor may have beneficial off-target effects [67]. Schoergenhofer et al. in *Clinical Science* showed that prasugrel, a potent P2Y₁₂

antagonist, does not attenuate the effect of LPS administration on markers of blood coagulation and platelet activation (P-selectin, soluble CD40, platelet-leukocyte complex) in human subjects [66]. They nonetheless showed that the LPS infusion increases histone-complexed DNA. Moreover, neither aspirin [68] nor GPIIb/IIIa antagonists [69] attenuate LPS induced platelet activation in a similar setting with human subjects. As Essex and Rao discussed [70], the effect of anti-platelet agents may become less discernible in conditions where LPS administration induce the production of TF from various cells such as monocytes and endothelial cells. The findings by Liverani et al. showed that P2Y₁₂-knockout mice had an unexpected enhancement of LPS-induced inflammation, corroborating data on the limited effect of TLR4 on platelets in LPS models [35,71].

***In vivo* LPS model**

In vivo, the effect of TLR ligands (TLRLs) on platelets tend to be strongly influenced by the indirect signals from other cells activated by TLRLs. Here we discuss only a couple of studies indicative of this problem. LPS can induce platelet sensitization for other agonists, platelet adhesion to blood vessels, and thrombocytopenia [72]. However, in *in vivo* LPS models, endothelial cells and neutrophils are also stimulated by LPS, and the relevance of platelet TLRs are less clear than in isolated platelets. In Rumbaut et al. 2006, LPS enhanced the formation of platelet thrombi in mouse cremaster venules, and this effect was mediated by TLR4 but was independent of neutrophils. Thus, the platelet-neutrophil complex was not important in this system. The authors suggested that the thrombus formation was dependent on microvascular endothelium [73]. Compared to thrombus formation, the role of platelet TLR4 in innate immunity and inflammation appears less clear. In Rumbaut et al. [73], LPS stimulation of platelets *ex vivo* failed to enhance platelet aggregation or P-selectin expression, in support of Cicala et al. [74]. A relatively limited direct effect of LPS on platelets was also suggested by Yin et al., in which LPS-induced microvascular thrombosis, thrombocytopenia, and mortality was mediated by vWF-binding function of GPIb-IX and GPIb-IX-mediated platelet adhesion to vascular wall structures, leading the authors to emphasize its role in the endothelium [75]. Intriguingly, TLR4 was sufficient for microvascular thrombosis. Stark et al. showed that transfusion of platelets from wild-type mice restored the responsiveness to LPS in TLR4-deficient mice with regards to microvascular thrombosis but not to plasma levels of TNF- α or IL-1 β [76]. These findings suggest that platelet TLR4 had a limited degree of effect on inflammation and innate immunity responses, but plays an important role in the formation of microvascular thrombus formation. However, the results with the LPS model in Stark et al. [76] significantly differ from Aslam et al., which showed that transfusion of platelets from wild-type mice into mice with defective TLR4 signaling was sufficient to induce increased serum TNF- α following LPS injection [77]. This difference adds to the aforementioned controversy on LPS effects on platelets [76].

TLR7 recognizes ssRNAs. Besides RNA viruses such as encephalomyocarditis virus (EMCV) and HIV, the small guanosine analogs such as loxoribine, imiquimod, and resiquimod (R848) activate TLR7. Both human and mouse platelets express a functional TLR7 [51]. Stimulation of this receptor by loxoribine and EMCV induces a platelet-neutrophil interaction and a reduction in platelet count in mice. TLR7 of platelets but not of neutrophils was the component that initiated interactions with neutrophils [51]. Isolated platelets showed Akt and p38 MAPK phosphorylation and increased expression of P-selectin and CD40L upon TLR7 stimulation. Of relevance to the platelet dichotomy, while TLR7 stimulation increased adherence to collagen, it was less pronounced compared to thrombin-stimulated platelets. Platelet aggregation was loose upon TLR7 stimulation compared to thrombin stimulation, leading the authors to consider that platelet TLR7 activation lacks prothrombotic properties. The molecular mechanistic detail causing such limited effects of TLR7 signaling requires further investigation, but it seems possible that TLR7 signaling may have an overall similarity with TLR2 signaling.

TLR9

Unlike nucleated cells in which the endosomal TLRs are restricted to the intracellular compartment, TLR3 and TLR9 can translocate to the surface upon platelet activation [52,78]. TLR9 recognizes DNA, and CpG-containing oligodeoxynucleotides are commonly used as TLR9 agonists. Strikingly, Panigrahi et al. reported that 2-(ω -carboxypropyl)pyrrole protein adducts (CAPs) are novel and unconventional ligands for TLR9 that accelerate thrombosis *in vivo* [79]. As CAPs are formed under vascular oxidative stress and belong to endogenous danger-associated molecular patterns (DAMPs), this study linked oxidative stress to innate immunity and thrombosis. Specifically, GP IIb/IIIa (integrin α IIB β 3) and P-selectin expression exhibited pronounced increases upon stimulation by CAPs, regardless of the parent proteins used for the CAP modifications, and this was mediated by TLR9. Human and murine platelets primed with low dose ADP and TRAP responded intensely to CAPs. The authors showed that Akt phosphorylation and Src family kinase play a role downstream of CAP/TLR9 signaling. However, it is poorly understood as yet whether and how surface-translocated TLR9 in platelets are activated during viral infections [49].

Conclusion and Perspectives

In this review, we focused on several informative studies in considering the functional versatility of platelets. By expressing a large number of receptors including TLRs, GPCRs, and tyrosine kinase-associated receptors including integrins, platelets are likely to differentiate between hemostatic and infectious stimuli [48]. In general, tyrosine kinase-based signaling, which is represented by insulin and growth hormone in endocrinology, is often involved in anabolism necessary for cell growth and division. By contrast, G-proteins appear to generally handle rapid and short time-

scale processes, as represented by epinephrine, glucagon (G_s), and muscarinic acetylcholine receptor (G_{q11}). In light of this, thrombin/PAR systems, which are G_q -coupled, might have evolved as a rapid pathway as rapid hemostasis is important upon severe injury. TLR signaling appears largely distinct from these pathways, although the involvement of PI3K/Akt and MAPK activation are observed in common with other signaling pathways. TLR signaling is generally slow and is not tightly linked to the Ca^{2+} or tyrosine kinases-mediated pathways. Therefore, a very simplified interpretation we conceive is as follows: during hemostasis, rapid G protein-PLC-IP₃-Ca²⁺ signaling contribute to rapid responses. This may proceed to a slow but extensive growth of thrombus dependent on GP IIb/IIIa, which is mainly associated with tyrosine kinase-mediated signaling. On the other hand, during innate immunity and inflammation, it is possible that a relatively limited number of pathways involving PI3K/Akt and other typical TLRs-associated pathways (e.g., IRAK-1 and NF- κ B) are employed, leading to slow responses limited in time length and extent. However, it is not clear to what extent the TLRs signaling pathways that have been established through studies with other cell types are used in platelets. For example, non-genomic functions of NF- κ B appear important in platelets, but largely remain unknown.

Of note, there has been a computational modeling study supporting the presence of a stepped hierarchy in physiological platelet responses [80]. Such studies have been motivated by the observation that thrombin, at different concentrations, can induce all hierarchies of responses ranging from mild integrin activation to procoagulant response (PS surface exposure), through the differential degrees of PAR activation. It is interesting to envisage that such hierarchical decision making can be extended for the TLR signaling system. Given the lack of involvement of intense PLC-IP₃-Ca²⁺ signaling, relatively low levels of cytoplasmic free Ca²⁺ may have a role in the decision making of platelets upon TLR signaling. However, such consideration needs more substantiation, given that TLR signaling in platelets have been poorly understood.

Although this article focused on dichotomy in terms of the proinflammatory and prothrombotic roles, platelets also has a dichotomy with respect to roles for increased vascular permeability upon inflammation as well as preservation of vascular barrier integrity and stabilization of endothelium [81].

It is possible that the dichotomous nature of platelets manifests as a versatility of platelets in the complex pathophysiology of sepsis. The current understanding of the pathophysiological and clinical significance of platelets in sepsis has been excellently reviewed and is beyond the scope of the present work [e.g., 82,83] Because of the lack of appreciable levels of cytokine receptor expression, platelets lack the ability to respond to cytokines. This feature may be useful to ensure versatile, localized, and microenvironment-dependent responses, rather than homogenous responses toward the systemic tone of inflammation. However, this uncorrelated feature is likely to jointly complicate analyses of how proinflammatory and thrombotic states of platelets associate with sepsis. Clearly, further analyses of

spatiotemporal features of platelet signaling pathways are warranted to better understand how platelets exert their versatility in diverse processes of sepsis.

References

1. Smyth SS, McEver RP, Weyrich AS, et al. (2009) Platelet functions beyond hemostasis. *J Thromb Haemost* 7(11): 1759-1766.
2. Thomas MR, Storey RF (2015) The role of platelets in inflammation. *Thromb Haemost* 114(3): 449-458.
3. Walsh TG, Metharom P, Berndt MC (2015) The functional role of platelets in the regulation of angiogenesis. *Platelets* 26(3): 199-211.
4. Li Z, Delaney MK, O'brien KA, et al. (2010) Signaling during platelet adhesion and activation. *Arterioscler Thromb Vasc Biol* 30(12): 2341-2349.
5. Durrant TN, van den Bosch MT, Hers I (2017) Integrin α Ib β 3 outside-in signaling. *Blood* 130(14): 1607-1619.
6. Yun SH, Sim EH, Goh RY, et al. (2016) Platelet activation: the mechanisms and potential biomarkers. *Biomed Res Int* 2016: 9060143.
7. Cimmino G, Golino P (2013) Platelet biology and receptor pathways. *J Cardiovasc Transl Res* 6(3): 299-309.
8. Smeeth L, Thomas SL, Hall AJ, et al. (2004) Risk of myocardial infarction and stroke after acute infection or vaccination. *N Engl J Med* 351: 2611-2618.
9. Delvaeye M, Conway EM. (2009) Coagulation and innate immune responses: can we view them separately? *Blood* 114(12): 2367-2374.
10. Lindmark E, Tenno T, Siegbahn A (2000) Role of platelet P-selectin and CD40 ligand in the induction of monocytic tissue factor expression. *Arterioscler Thromb Vasc Biol* 20(10): 2322-2328.
11. Grover SP, Mackman N (2018) Tissue factor: An essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol* 38(4): 709-725.
12. Bernardo A, Ball C, Nolasco L, et al. (2004) Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultra large von Willebrand factor multimers under flow. *Blood* 104(1): 100-106.
13. Engelmann B, Massberg S (2013) Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 13(1): 34-45.
14. Claushuis TAM, Stoppelaar SF, Stroo I, et al. (2017) Thrombin contributes to protective immunity in pneumonia-derived sepsis via fibrin polymerization and platelet-neutrophil interactions. *J Thromb Haemost* 15(4): 744-757.
15. Venkata C, Kashyap R, Farmer JC, et al. (2013) Thrombocytopenia in adult patients with sepsis: Incidence, risk factors, and its association with clinical outcome. *J Intensive Care* 1(1): 9.
16. Burstein SA (1997) Cytokines, platelet production and hemostasis. *Platelets* 8(2-3): 93-104.
17. Esmon CT (2005) The interactions between inflammation and coagulation. *Br J Haematol* 131(4): 417-430.
18. Peerschke EI, Reid KB, Ghebrehiwet B (1993) Platelet activation by C1q results in the induction of alpha IIb/beta 3 integrins (GPIIb-IIIa) and the expression of P-selectin and procoagulant activity. *J Exp Med* 178(2): 579-587.
19. Sims PJ, Faioni EM, Wiedmer T, et al. (1988) Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *J Biol Chem* 263(34): 18205-18212.
20. Falati S, Liu Q, Gross P, et al. (2003) Accumulation of tissue factor into developing thrombi *in vivo* is dependent upon microparticle P-selectin glycoprotein 1 and platelet P-selectin. *J Exp Med* 197(11): 1585-1598.
21. Müller F, Mutch NJ, Schenk WA, et al. (2009) Platelet polyphosphates are proinflammatory and procoagulant mediators *in vivo*. *Cell* 139(6): 1143-1156.
22. Golias C, Charalabopoulos A, Stagikas D, et al. (2007) The kinin system-bradykinin: biological effects and clinical implications. Multiple role of the kinin system-bradykinin. *Hippokratia* 11(3): 124-128.
23. Chatterjee M, Huang Z, Zhang W, et al. (2011) Distinct platelet packaging, release, and surface expression of proangiogenic and antiangiogenic factors on different platelet stimuli. *Blood* 117(14): 3907-3911.
24. Pagel O, Walter E, Jurk K et al. (2017) Taking the stock of granule cargo: platelet releasate proteomics. *Platelets* 28(2): 119-128.
25. Page CP (1988) The involvement of platelets in non-thrombotic processes. *Trends Pharmacol Sci* 9(2): 66-71.
26. Amison RT, Momi S, Morris A, et al. (2015) RhoA signaling through platelet P2Y1 receptor controls leukocyte recruitment in allergic mice. *J Allergy Clin Immunol* 135(2): 528-538.
27. Pitchford SC, Yano H, Lever R, et al. (2003) Platelets are essential for leukocyte recruitment in allergic inflammation. *J Allergy Clin Immunol* 112(1): 109-118.
28. Ind PW (1991) Platelet and clotting abnormalities in asthma. *Clin Exp Allergy* 21(4): 395-398.
29. Gachet C (2001) ADP receptors of platelets and their inhibition. *Thromb Haemost* 86(01): 222-232.
30. Gachet C (2001) Identification, characterization, and inhibition of the platelet ADP receptors. *International journal of hematology* 74(4): 375.
31. Dorsam RT, Kunapuli SP (2004) Central role of the P2Y12 receptor in platelet activation. *J Clin Invest* 113(3): 340-345.
32. Léon C, Ravanat C, Freund M, et al. (2003) Differential involvement of the P2Y1 and P2Y12 receptors in platelet procoagulant activity. *Arterioscler Thromb Vasc Biol* 23(10): 1941-1947.
33. Amison RT, Jamshidi S, Rahman KM, et al. (2018) Diverse signalling of the platelet P2Y1 receptor leads to a dichotomy in platelet function. *Eur J Pharmacol* 827: 58-70.
34. Amison RT, Arnold S, O'Shaughnessy BG, et al. (2017) Lipopolysaccharide (LPS) induced pulmonary neutrophil recruitment and platelet activation is mediated via the P2Y1 and P2Y14 receptors in mice. *Pulm Pharmacol Ther* 45: 62-68.
35. Liverani E, Rico MC, Yaratha L, et al. (2014) LPS-induced systemic inflammation is more severe in P2Y12 null mice. *J Leukoc Biol* 95(2): 313-323.

36. Diehl P, Olivier C, Halscheid C, et al. (2010) Clopidogrel affects leukocyte dependent platelet aggregation by P2Y12 expressing leukocytes. *Basic Res Cardiol* 105(3): 379-387.
37. Wang L, Jacobsen SE, Bengtsson A, et al. (2004) P2 receptor mRNA expression profiles in human lymphocytes, monocytes and CD34+ stem and progenitor cells. *BMC Immunol* 5: 16.
38. Paruchuri S, Tashimo H, Feng C, et al. (2009) Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. *J Exp Med* 206(11): 2543-2555.
39. Pleines I, Hagedorn I, Gupta S, et al. (2012) Megakaryocyte-specific RhoA deficiency causes macrothrombocytopenia and defective platelet activation in hemostasis and thrombosis. *Blood* 119(4): 1054-1063.
40. Devreotes P, Horwitz AR (2015) Signaling networks that regulate cell migration. *Cold Spring Harbor perspectives in biology* 7(8): a005959.
41. Vogt S, Grosse R, Schultz G, et al. (2003) Receptor-dependent RhoA Activation in G12/G13-deficient Cells genetic evidence for an involvement of Gq/G11. *J Biol Chem* 278(31): 28743-28749.
42. Bartolomé RA, Wright N, Molina-Ortiz I, et al. (2008) Activated Gα13 Impairs Cell Invasiveness through p190RhoGAP-Mediated Inhibition of RhoA Activity. *Cancer research* 68(20): 8221-8230.
43. Aslan JE, McCarty OJT (2013) Rho GTPases in platelet function. *J Thromb Haemost* 11(1): 35-46.
44. Kozasa T, Jiang X, Hart MJ, et al. (1998) p115 RhoGEF, a GTPase activating protein for Gα12 and Gα13. *Science* 280(5372): 2109-2111.
45. Gong H, Shen B, Flevaris P, et al. (2010) G protein subunit Gα13 binds to integrin αIIbβ3 and mediates integrin "outside-in" signaling. *Science* 327(5963): 340-343.
46. Arthur WT, Petch LA, BurrIDGE K (2000) Integrin engagement suppresses RhoA activity via a c-Src-dependent mechanism. *Curr Biol* 10(12): 719-722.
47. Gao J, Zoller KE, Ginsberg MH, et al. (1997) Regulation of the pp72syk protein tyrosine kinase by platelet integrin αIIbβ3. *EMBO J* 16(210): 6414-6425.
48. Cognasse F, Nguyen KA, Damien P, et al. (2015) The inflammatory role of platelets via their TLRs and Siglec receptors. *Front Immunol* 6: 83.
49. Hottz ED, Bozza FA, Bozza PT (2018) Platelets in immune Response to virus and immunopathology of viral infections. *Front med* 5: 121.
50. Ward JR, Bingle L, Judge HM, et al. (2005) Agonists of toll-like receptor (TLR) 2 and TLR4 are unable to modulate platelet activation by adenosine diphosphate and platelet activating factor. *Thromb Haemost* 93(4): 831-838.
52. Koupnova M, Vitseva O, MacKay CR, et al. (2014) Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* 124(5): 791-802.
53. Thon JN, Peters CG, Machlus KR, et al. (2012) T granules in human platelets function in TLR9 organization and signaling. *J Cell Biol* 198(4): 561-574.
54. D'Atri LP, Etulain J, Rivadeneyra L, et al. (2015) Expression and functionality of toll-like receptor 3 in the megakaryocytic lineage. *J Thromb Haemost* 13(5): 839-850.
55. Blair P, Rex S, Vitseva O, et al. (2009) Stimulation of Toll-like receptor 2 in human platelets induces a thromboinflammatory response through activation of phosphoinositide 3-kinase. *Circ Res* 104(3): 346-354.
56. Rex S, Beaulieu LM, Perlman DH, et al. (2009) Immune vs. Thrombotic stimulation of platelets differentially regulates signaling pathways, intracellular protein-protein interactions, and α-granule release. *Thromb Haemost* 102(1): 97-110.
57. Takashima N, Fujioka A, Hayasaka N, et al. (2006) Gq/11-induced intracellular calcium mobilization mediates Per2 acute induction in Rat-1 fibroblasts. *Genes Cells* 11(9): 1039-1049.
58. Nicu EA, van der Velden U, Nieuwland R, et al. (2009) Elevated platelet and leukocyte response to oral bacteria in periodontitis. *J Thromb Haemost* 7(1): 162-170.
59. Assinger A, Laky M, Schabbauer G, et al. (2011) Efficient phagocytosis of periodontopathogens by neutrophils requires plasma factors, platelets and TLR2. *J Thromb Haemost* 9(4): 799-809.
60. Resendiz JC, Kroll MH, Lassila R (2007) Protease-activated receptor-induced Akt activation-regulation and possible function. *J Thromb Haemost* 5(12): 2484-2493.
61. Keane C, Tilley D, Cunningham A, et al. (2010) Invasive *Streptococcus pneumoniae* trigger platelet activation via Toll-like receptor 2. *J Thromb Haemost* 8(12): 2757-2765.
62. Kälvegren H, Skoglund C, Helldahl C, et al. (2010) Toll-like receptor 2 stimulation of platelets is mediated by purinergic P2X1-dependent Ca²⁺ mobilisation, cyclooxygenase and purinergic P2Y1 and P2Y12 receptor activation. *Thromb Haemost* 103(02): 398-407.
63. Rivadeneyra L, Carestia A, Etulain, J, et al. (2014) Regulation of platelet responses triggered by Toll-like receptor 2 and 4 ligands is another non-genomic role of nuclear factor-kappaB. *Thromb Res* 133(2): 235-243.
64. Lopes-Pires ME, Clarke SR, Marcondes S, et al (2017) Lipopolysaccharide potentiates platelet responses via toll-like receptor 4-stimulated Akt-Erk-PLA2 signaling. *PloS one* 12(11): e0186981.
65. Thomas MR, Outteridge SN, Ajjan RA, et al. (2015) Platelet P2Y12 inhibitors reduce systemic inflammation and its prothrombotic effects in an experimental human model. *Arterioscler Thromb Vasc Biol* 35(12): 2562-2570.
66. Tunjungputri RN, Ven AJ Van Der, Riksen N, et al. (2015) Differential effects of platelets and platelet inhibition by ticagrelor on TLR2-and TLR4-mediated inflammatory responses. *Thromb Haemost* 113(5): 1035-1045.
67. Schoergenhofer C, Schwameis M, Hobl EL, et al. (2016) Potent irreversible P2Y12 inhibition does not reduce LPS-induced coagulation activation in a randomized, double-blind, placebo-controlled trial. *Clin Sci* 130(6): 433-440.
68. Sumaya W, Storey RF (2017) Ticagrelor: Effects beyond the P2Y12 receptor. *Interv Cardiol Clin* 6(1): 49-55.
69. Pernerstorfer T, Stohlawetz P, Hollenstein U, et al. (1999) Endotoxin-induced activation of the coagulation cascade in humans: effect of acetylsalicylic acid and acetaminophen. *Arterioscler Thromb Vasc Biol* 19(10): 2517-2523.

70. Derhaschnig U, Pachinger C, Schweeger-Exeli I, et al. (2003) Blockade of GPIIb/IIIa by eptifibatid and tirofiban does not alter tissue factor induced thrombin generation in human endotoxemia. *Thromb Haemost* 90(6): 1054-1060.
71. Essex DW, Rao AK (2016) P2Y12 receptor inhibition and LPS-induced coagulation. *Clin Sci* 130(6): 441-442.
72. Akinosoglou K, Theodoraki S, Xanthopoulou I, et al. (2017) Platelet reactivity in sepsis syndrome: results from the PRESS study. *Eur J Clin Microbiol Infect Dis* 36(12): 2503-2512.
73. Shibazaki M, Kawabata Y, Yokochi T, et al. (1999) Complement-dependent accumulation and degradation of platelets in the lung and liver induced by injection of lipopolysaccharides. *Infect Immun* 67(10): 5186-5191.
74. Rumbaut RE, Bellera RV, Randhawa JK, et al. (2006) Endotoxin enhances microvascular thrombosis in mouse cremaster venules via a TLR4-dependent, neutrophil-independent mechanism. *Am J Physiol Heart Circ Physiol* 290(4): H1671-H1679.
75. Cicala C, Santacrose C, Itoh H, et al. (1997) A study on rat platelet responsiveness following intravenous endotoxin administration. *Life Sci* 60(2): PL31-PL38.
76. Yin H, Stojanovic-Terpo A, Xu W, et al. (2013) Role for Platelet Glycoprotein Ib-IX and Effects of its Inhibition in Endotoxemia-Induced Thrombosis, Thrombocytopenia, and Mortality Significance. *Arterioscler Thromb Vasc Biol* 33(11): 2529-2537.
77. Stark RJ, Aghakasiri N, Rumbaut RE (2012) Platelet-derived Toll-like receptor 4 (Tlr-4) is sufficient to promote microvascular thrombosis in endotoxemia. *PloS one* 7(7): e41254.
78. Aslam R, Speck ER, Kim M, et al (2006) Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor- α production *in vivo*. *Blood* 107(2): 637-641.
79. Blasius AL, Beutler B (2010) Intracellular toll-like receptors. *Immunity* 32(3): 305-315.
80. Panigrahi S, Ma Y, Hong L, et al. (2012) Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyper-reactivity and thrombosis. *Circ Res* 112(1): 103-112.
81. Sveshnikova AN, Ataulakhanov FI, Panteleev MA (2015) Compartmentalized calcium signaling triggers subpopulation formation upon platelet activation through PAR1. *Mol Biosyst* 11(4): 1052-1060.
82. Mammoto T, Jiang A, Jiang E, et al. (2015) Platelet-rich plasma extract prevents pulmonary edema through angiopoietin-Tie2 signaling. *Am J Respir Cell Mol Biol* 52(1): 56-64.
83. Dewitte A, Lepreux S, Villeneuve J, et al. (2017) Blood platelets and sepsis pathophysiology: A new therapeutic prospect in critical ill patients? *Ann Intensive Care* 7(1): 115.
84. Larkin CM, Santos-Martinez MJ, Ryan T, et al. (2016) Sepsis-associated thrombocytopenia. *Thromb Res* 141: 11-16.

***Corresponding author:** Kazuhisa Nishizawa, Teikyo University School of Medical Technology, Kaga, Itabashi, Tokyo, 173-8605 Japan, Tel: +81-3-3964-1211, Fax: +81-3-5944-3354; Email: kazunet@med.teikyo-u.ac.jp

Received date: June 14, 2018; **Accepted date:** June 21, 2018; **Published date:** June 25, 2018

Citation: Seki R, Nishizawa K (2018) Inflammatory vs. Thrombotic States of Platelets: A Mini-review with a Focus on Functional Dichotomy. *Ann Biomed Res* 1(2): 107.

Copyright: Seki R, Nishizawa K (2018) Inflammatory vs. Thrombotic States of Platelets: A Mini-review with a Focus on Functional Dichotomy. *Ann Biomed Res* 1(2): 107.