DOI: 10.1002/JLB.MR0618-213R

JLB JOURNAL OF LEUKOCYTE BIOLOGY

REVIEW

Platelet TLR4 at the crossroads of thrombosis and the innate immune response

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Abstract

Platelet TLR-4 activation by pathogen- or damage-associated molecular pattern molecules triggers pro-thrombotic, proinflammatory, and pro-coagulant effector responses. Moreover, platelet TLR4 has a prominent role as a sensor of high lipopolysaccharide circulating levels during sepsis and in the clearance of pathogens mediated by neutrophils. This review presents evidence pointing to TLR4 as a bridge connecting thrombosis and innate immunity.

KEYWORDS

HMGB-1, lps, nets, platelets, sepsis, thrombosis, TLRs

1 | INTRODUCTION

TLRs, usually expressed by immune cells, can activate innate and adaptive immune defenses by identifying pathogen- or damage-associated molecular patterns (PAMPs and DAMPs, respectively). Till date, 10 functional TLRs (i.e., TLR1-TLR10) in humans and 13 active TLRs in laboratory mice have been identified. Each member of the TLR family recognizes distinct PAMPs. TLR1, 2, 4, 5, and 6 are engaged in the recognition of bacterial PAMPs such as LPS, peptidoglycan, lipoprotein, and flagellin. TLR3, 7, 8, and 9 recognize nucleotide derivatives and TLR10 tryacilated lipopeptides.[1]

Besides having a crucial role in hemostasis and thrombosis, platelets significantly contribute to amplifying the inflammatory and immune response elicited by neutrophils and monocytes against infectious or sterile insults.[2] The expression of functional TLRs is one of the mechanisms through which platelets participate in the immune response. Although platelets express most members of the TLR family, TLR4 is one of the most characterized not only because Gram-negative bacterial infection is a major clinical problem with few therapeutic options, but also because platelets significantly contribute to the pathophysiology of sepsis and are associated with the high mortality rates observed with this disease[3–5]

Abbreviations: ACS, Acute coronary syndrome; ARB, Angiotensin II receptor blocker; DAMPs, Damage-associated molecular patterns; DIC, Disseminated intravascular coagulation; Fn-EDA +, Fibronectin containing extra domain A; GC, Guanylyl cyclase; HMGB1, High-mobility group box 1; HPU, *H. pylori* urease; NETs, Neutrophil extracellular traps; PAMPs, Pathogen-associated molecular patterns; PF4, Platelet factor 4; SNARE, Soluble N-ethylmaleimide-sensitive factor attachment protein receptors; TF, Tissue factor; TF-PCA, Tissue factor-pro-coagulant activity; TRIF, Toll-interleukin-1 receptor domain-containing adapter inducing IFN-β; TXA2, Thromboxane A2; VWF, von Willebrand Factor

2 | PLATELET TLR4 ACTIVATION PROMOTES PLATELET PRO-THROMBOTIC AND PRO-COAGULANT RESPONSES

The scientific interest in the expression and functionality of platelet TLR4 started with the aim of understanding not only the pathogenic mechanisms of thrombocytopenia and thrombotic events during sepsis, but also the relationship between platelets in infectious inflammation and atherosclerotic vascular diseases.

In 2004, Shiraki et al. first determined that human platelets express functional TLRs.[6] However, they were not able to detect TLR4. Later on, it was shown that human and murine platelets express TLR4, and that it is a functional receptor that contributes to thrombocytopenia via neutrophil-dependent pulmonary sequestration in response to LPS.[7] These findings were simultaneously confirmed by Garraud et al., who demonstrated the expression of several members of the TLR family including TLR4 in human platelets and its down-regulation by LPS-mediated platelet activation.[8] Since then, several groups have explored the in vitro effect of platelet TLR4 activation by LPS; however, there are still many controversies in the field.[9]

Whereas some studies have shown that TLR4 activation by LPS induces platelet activation, most observed that this is not a direct effect but rather potentiates platelet responses elicited by classical agonists including aggregation, ATP release, P-selectin expression, and the formation of mixed aggregates between platelets and neutrophils.[10–13] It is worth mentioning that, among these studies, only the experiments by Nocella et al.[13] were performed using LPS in the picogram range; all other studies required 10 times more LPS to achieve similar results. The use of such a high LPS concentration raises concerns as it is considered that these LPS levels are not

Received: 28 August 2018 | Revised: 2 November 2018 | Accepted: 19 November 2018

representative of the concentrations observed in the clinical setting (approx. 100 pg/ml). However, it is conceivable that, in the local infected area, these values can be much higher and therefore the effect of this concentration of LPS on platelets might be relevant. In contrast to these findings, it has also been reported that stimulation of platelets with LPS results in platelet inhibition.[14,15]

Besides eliciting pro-thrombotic responses, platelet activation with LPS also induces platelet-dependent tissue factor pro-coagulant activity (TF-PCA) and prompts faster thrombin generation in a TLR4-dependent manner.[16] Although TF expression has been mainly ascribed to neutrophils and monocytes/macrophages,[17] considering the high number of circulating platelets, it seems reasonable to consider that platelet-mediated TF formation by LPS activation represents a relevant source of TF during sepsis. Unlike aggregation or granule secretion, TF-PCA activity is directly induced by LPS and requires TF pre-mRNA splicing. Induction of splicing and post-transcriptional TF expression were also observed when platelets were incubated with *Escherichia coli*, indicating that the splicing-dependent pro-coagulant activity of platelets may occur if LPS is released into the blood systemically, locally or potentially by direct encounters between platelets and Gram-negative pathogens.[16] Matus et al. observed the ability of *Escherichia coli* strain O111 to induce TF-dependent thrombin generation in platelets. However, they were unable to observe the same effect when platelets where stimulated with ultra-pure *Escherichia coli* 0111 LPS.[18]

The reasons for these discrepancies in pro-thrombotic and pro-coagulant responses elicited by LPS-stimulated platelets are not clear, but they may be associated with the presence or not of co-factors, the source and purity of the LPS employed, incubation time, and the intersubject variability of platelet responses. LPS-mediated activation involves interactions not only with the transmembrane signaling receptor TLR4 but also with CD14 displayed on the cell surface. Platelets express TLR4 in sufficient quantities to physically bind LPS, but they do not express CD14.[19,20] Therefore, if platelet activation triggered by LPS is performed in washed platelets, the addition of plasma or serum (containing microgram levels of soluble CD14) or recombinant CD14 is required. Interestingly, it was recently demonstrated that *Helicobacter pylori* urease (HPU) activates platelets and also increases the levels of mRNA encoding IL-1β and CD14.[21] This finding may be crucial to explaining platelet activation during sepsis, as it indicates that although CD14 is not present under physiologic conditions, it can be up-regulated during infection. Moreover, similar to TF but promoting inflammation, IL-1β generation upon platelets LPS-stimulation during sepsis, can significantly contribute to the burden of systemic IL-1β levels produced by neutrophils and monocytes/macrophages.

Regarding the source of LPS, not all LPS preparations have similar effectiveness with regard to inducing platelet activation. For example, the ability in potentiating collagen-induced platelet aggregation varies with the source of LPS, in the order of *Escherichia coli* 0127:B8 > 0111:B4 > 055:B5[10].

In addition, it becomes important to differentiate the effects of LPS on platelets depending on the specie the endotoxin was originated. Berthet et al., found that *Salmonella minnesota* LPS is a more potent inductor of soluble CD62p and CD40L as well as platelet-derived growth factor compared to *Escherichia coli* LPS.[22] Moreover, platelet supernatant obtained from *Salmonella minnesota* LPS-stimulated platelets is the only one capable of inducing IL-6, TNF-α, and IL-8 release from peripheral blood mononuclear cells. On the other hand, Claushuis et al., studied the direct effect on platelet activation of LPS derived from *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* finding no evidence of platelet activation except for slightly higher phosphatidylserine positivity after *Escherichia coli* LPS stimulation.[23] Interestingly, this study also showed that *Klebsiella pneumoniae* did not induce classical platelet activation but it increased platelet mitochondrial respiration. Therefore, it is clear that not all LPS exert similar responses and more comparisons studies are necessary to clarify the conflictive data regarding the effect of LPS on platelets.

In addition, and regrettably, many studies neither provide information on the bacterial strain of origin, nor on the purity of the used LPS. Although some groups have used homemade LPS where the purity level has not been determined[12,18] others used commercial LPS, where even though purity level is indicated, even within the same brand, different purification techniques are applied possibly giving variable results regarding purity and potency of the endotoxin.[10,11] One of the pitfalls using LPS of unknown or insufficient purity is that it might activate other receptors (especially TLR2) instead of TLR4. Despite these differences in the experimental procedures overall, the available data indicate that the activation of platelet TLR4 by LPS can result in platelet activation, especially in synergism with other platelet agonists, a condition particularly present during sepsis. Depending on the complex interplay between the coagulation, immune, and fibrinolytic systems that take place during sepsis, platelet activation triggered by LPS may result in either thrombocytopenia or thrombotic events, as occurring during disseminated intravascular coagulation (DIC), which is a frequent consequence of sepsis and is associated with organ failure. Although the pathogenic mechanisms of DIC are still not completely elucidated, a hyper-coagulation state and circulating activated platelets are considered to contribute to vascular occlusion. Interestingly, platelet TLR4 has been shown to be sufficient to accelerate microvascular thrombosis in the cremaster venules during endotoxemia, suggesting that the presence of TLR4 on platelets could be a link between DIC and sepsis.[24] In contrast, recent evidence has demonstrated that mice deficient in platelet MyD88, the TLR common adaptor protein (with the exception of TLR3), does not show hallmark sepsis responses such as thrombocytopenia, coagulation, endothelial activation, or distant organ injury in response to *Klebsiella pneumoniae*. These results suggested that platelet MyD88-dependent TLR signaling does not contribute to the host response during Gram-negative sepsis with the exception of a moderate decrease in TNF-α and keratinocyte chemoattractant production.[25] Thus, it is clear that more studies are required to determine whether the reported effects are specific for *Klebsiella pneumoniae* or shared by other Gram-negative bacteria as well as by LPS.

TLR4 ligands include not only PAMPs but also DAMPs.[26] Among the latter, high-mobility group box 1 (HMGB1) has been lately the focus of attention as it is a strong inductor of platelet activation. It appears to

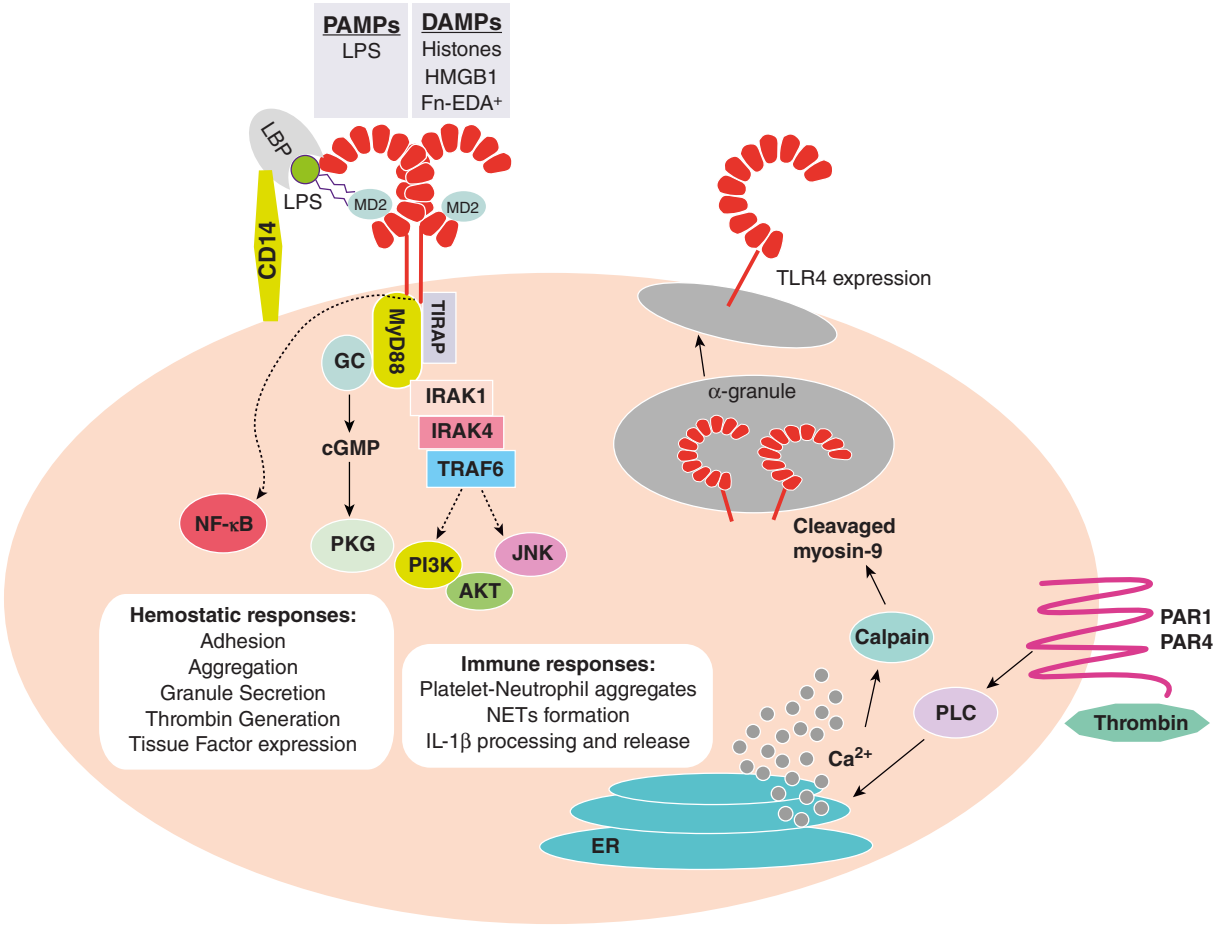

FIGURE 1 **Platelet TLR4-mediated effector responses**. Activation of platelet TLR4 by either PAMPs or DAMPs triggers platelet hemostatic, and immune responses. The signaling pathways involved include the activation of MyD88, PI3K, NF-κB, and PKG. Thrombin activation up-regulates TLR4 expression through PAR/PLC pathway, calcium, and calpain

be a relevant molecule involved in coagulation abnormalities and organ injury observed in hemorrhagic shock and resuscitation.[27] Additionally, cellular fibronectin containing extra domain A (Fn-EDA +), which is produced in response to tissue injury in several disease states, has pro-thrombotic activity, as it interacts with platelet TLR4 and promotes agonist-induced platelet aggregation.[28] Interestingly, histones, other DAMPs bound to neutrophil extracellular traps, (NETs) trigger both pro-thrombotic and pro-coagulant platelet-mediated responses, partly by interacting with TLR4[29,31] (Fig. 1). As discussed below, this effect is critical to creating a positive feedback regulatory system between neutrophils and platelets during the immune response, not only during infectious but also during sterile inflammatory conditions.[32]

3 | PLATELET TLR4 EXPRESSION AND SIGNAL TRANSDUCTION

Platelet activation with LPS results in the down-regulation of platelet TLR4[8]. Interestingly, and in contrast, the levels of TLR4 on the platelet membrane are significantly increased upon platelet stimulation with thrombin.[33] The explanation for these opposing effects is not clear. The molecular mechanism for the up-regulation of TLR4 mediated by thrombin involves activation of the PLC-calpain-myosin 9-Rab7b axis, which regulates TLR4 trafficking from alpha granules to the platelet membrane.[33] Thus, it could be possible that the interaction between LPS and platelet TLR4 does not trigger this signaling pathway.

The mechanism by which TLR4 transduces the interaction with its ligands in an anucleated platelet has not been completely elucidated. In nucleated cells, LPS-induced cellular activation through TLR4/MD2 is complex as many signaling elements are involved. However, there are two distinct initiation points in the signaling process, depending upon the recruitment of the adaptor proteins MyD88 or Toll-interleukin-1 receptor domain-containing adapter inducing IFN-β (TRIF).[34] The MyD88-dependent pathway leads to early activation of the transcription factor NF-κB, the production of proinflammatory cytokines such as TNF-α, and Th1 cell responses. The TRIF-dependent pathway induces phosphorylation and dimerization of TRIF-3, resulting in Interferon gamma production. So far, in anucleated platelets, only the MyD88 including activation of the NF-κB pathway appears to be involved in LPS-mediated activation. In this sense, murine and human platelets have the molecular machinery necessary for signal transduction downstream of TLR4, through the MyD88-IKK-NF-κB pathway, which has been shown to participate in platelet functional responses.[7,10,35] We have observed that activation of human platelets with LPS results in the phosphorylation of NF-κB and degradation of its inhibitor. Moreover, pre-incubation of platelets with unrelated

specific inhibitors of NF-κB inhibits the potentiation of thrombin-induced platelet activation responses mediated by LPS suggesting that nongenomic effects of NF-κB pathway activation are involved in LPS-induced platelet activation.[11] Notably, some of the inhibitors used to block NF-κB in platelets (i.e., BAY 11–7082) suppress IKK-β activity; this kinase is involved in the phosphorylation of soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), particularly platelet SNAP23.[36] Because SNARE complex formation is involved in platelet secretion, it could be conceivable that the NF-κB pathway controls platelet secretion mediated by LPS, in particular when acting in combination with subthreshold thrombin concentrations. The observation that platelet stimulation with LPS plus thrombin or collagen triggers PI3K/AKT activation[12] and that AKT phosphorylates IKK-β renders IKK-β a relevant potential role as a mediator of LPS-induced platelet secretion. Moreover, this hypothesis appears to be more comprehensive as other TLR4 ligands such as cellular Fn-EDA + potentiates thrombin-induced IKK/NF-κB activation in WT but not in TLR4-/- platelets.[28]

Conversely, the LPS priming of thrombin-mediated platelet aggregation and secretion has also been shown to be coupled to cGMP-dependent signaling.[10] A similar effect was described for platelet activation or priming by the DAMP molecule HMGB1. Specifically, platelet activation with HMGB1 is mediated via TLR4 and MyD88-dependent recruitment of platelet guanylyl cyclase (GC) toward the plasma membrane, followed by MyD88/GC complex formation and activation of cGMP-dependent protein kinase.[37] Interestingly and intriguingly, the cGMP activation pathway in platelet biology has a biphasic response. Whereas low and rapid increases in cGMP levels induce platelet activation, the opposite effect is achieved by sustained and higher levels of cGMP.[38] The role of cGMP in TLR4-mediated platelet responses was further expanded by Yang et al., who found that NF-κB is also involved in the TLR4/MyD88 and cGMP/PKG pathways. Remarkably, and in contrast with previous studies, they observed that cGMP levels decreased upon HMGB1-mediated TLR4 stimulation[39] (Fig. 1). Undeniably, cGMP/PKG appears to be a potential final downstream signaling pathway involved in platelet TLR4 transduction signaling, triggered either by PAMPs or DAMPs. However, due to the current controversy, more data from independent groups are necessary to elucidate the role of sGC/cGMP/PKG and its potential relationship with the NF-κB pathway in platelet responses triggered by TLR4 activation. Moreover, a recent study using two different NF-κB reporter cell lines and TLR4-green fluorescent protein, showed that in contrast to cell activation with LPS stimulation with HMGB1 decreased NF-κB activity questioning the specificity of HMGB1 as a ligand for TLR4.[40] Thus, it is clear that TLR4 ligands and downstream signaling in anucleated platelets, is still a puzzle does need to be solved.

4 | ROLE OF PLATELET TLR4 IN THE IMMUNE RESPONSE

Neutrophils are the first line of defense against pathogens. Besides phagocytosis and degranulation, a third strategy to combat microbes is the formation of NETs.[41] Very soon after the discovery of NETs and using a mouse model of sepsis, it was shown that platelets are required to fuel the formation of NETs. During severe sepsis, platelet TLR4 senses the high levels of circulating LPS and induces platelet binding to adherent neutrophils in the capillaries of the lungs and liver, which not only induces thrombocytopenia but also leads to robust neutrophil activation and the formation of NETs, thereby increasing the capacity of the immune system to trap and kill circulating bacteria.[42] This seminal study not only demonstrated the in vivo functional role of TLR4, but also introduced the original concept that platelets function as a barometer for systemic infection and facilitate the ensnaring of bacteria in the circulation. This novel property of platelets has also been observed in human platelets.[43–45]

How do platelets promote NET formation? We found that platelet stimulation with LPS triggers the synthesis and/or secretion of von Willebrand factor (VWF), platelet factor 4 (PF4), and thromboxane A2 (TXA_2).[44] The observation that inhibition of their action markedly decreases the release of DNA traps and that recombinant PF4 is a strong NET inducer further indicated that these three molecules are mediators of NET formation induced by platelets. Moreover, the platelet and neutrophil receptors involved in NET formation include GP Ib in platelets and β2-integrin in neutrophils. Because GPIb and β2 integrin are VWF receptors, we suggest that once TXA_2 is generated upon LPS stimulation of platelets, it induces the release of VWF and PF4 from alpha granules. VWF binds to GPIb and CD11b (β2 integrin), acting as a bridge that brings platelets and neutrophils into proximity. Simultaneously, PF4 binds to glycosaminoglycans on the neutrophil surface and triggers the release of DNA traps.[44] Besides LPS, activated platelets release and present HMGB1 to neutrophils and commit them to autophagy and NET generation.[43] Therefore, platelet TLR4 appears to be a critical receptor by which platelets sense and recognize microbes, respond to them, and participate in the clearance of bacteria and other pathogens.

Interestingly, the story does not end here. Once NETs are formed, they in turn activate circulating platelets and facilitate their recruitment and activation by presenting histones to platelet TLR2 and TLR4. We have shown that all histones are substrates for platelet adhesion and spreading. They also trigger fibrinogen binding, aggregation, VWF release, P-selectin, and phosphatidylserine exposure and the formation of platelet-leukocyte aggregates.[30] Moreover, histones induce a platelet pro-coagulant phenotype as they induce thrombin generation, partly by the activation of TLR2 and TLR4[29]. This intricate interplay between neutrophils and platelets led to coin the term "immunothrombosis," which describes an innate immune response induced by the formation of thrombi in microvessels and supported by immune cells together with specific thrombosis-related molecules. Immunothrombosis facilitates the recognition, entrapment, and disposal of pathogens, thereby protecting host integrity.[46] However, it is important to remember that too much of a good thing is never good. If these reactions occur systemically and are sustained for a long period, as observed in sepsis, they can lead to DIC followed by multiorgan failure and even death. In addition, under sustained sterile inflammatory conditions, they can be involved in the pathogenesis of both arterial or venous thrombotic diseases as well as autoimmune-related disorders such as lupus, arthritis, or vasculitis.[41,45]

5 | RELEVANCE OF PLATELET TLR4 IN THE CLINIC

Clinical studies related to the role of platelet TLR4 are just emerging. The first epidemiologic study performed in a large community-based cohort (1,625 participants in the Framingham Heart Study) showed that platelets express all TLR transcripts, that they are more abundant in women, and have distinct associations with cardiovascular risk and inflammatory biomarkers that vary with sex.[47] Interestingly, these cardiovascular, sex-dependent differences associated with TLR4 genetic variants have been previously described. Specifically, an association has been reported between the Asp299Gly TLR4 gene polymorphism with acute coronary events in men but not in women.[48] In addition, there is evidence showing that TLR4 is associated with the initiation, progression, and eventual rupture of the atheroma plaque.[49] Although these studies have mainly looked at TLR4 in monocytes and T lymphocytes, evidence showing that PAMPs and DAMPs interact with platelet TLR4 and trigger platelet activation clearly indicates that increased expression and/or functionality of platelet TLR4 might be involved in the pathogenesis of atherosclerosis and acute coronary events. In this sense, it has recently been reported that patients with unstable angina pectoris have enhanced expression of both platelet TLR2 and TLR4 compared to patients with stable angina pectoris and subjects with normal coronary arteries. In addition, both TLRs were found to positively correlated with the peak of troponin-T levels in patients with acute coronary syndrome (ACS), suggesting that platelet TLR expression is also associated with the severity of myocardial infarction.[50] Interestingly, the observation that TLR2 and TLR4 expression is greater in patients with stable angina pectoris compared to patients with normal coronary arteries suggests that TLR expression might play a role in atherogenesis, beyond thrombosis leading to ACS. Another study has shown that although TLR4 is significantly up-regulated in patients with acute myocardial infarction, activation of these platelets with LPS exerts similar effector responses as those observed in platelets from healthy donors.[51] Whether the activation of platelet TLR4 has a major role in acute cardiovascular events or during atherosclerosis development and whether platelet TLR4 activation has a different role to that of immune cells are very interesting issues that still require further investigation. Moreover, another relevant point that remains to be explored is if the eventual participation of platelet TLR4 in ACS or atherosclerosis is linked to increased levels of DAMPs, PAMPs, or both.

Regarding sepsis, it has been found that septic and thrombocytopenic patients at admission have increased levels of platelet TLR4 and PAC-1 compared to nonthrombocytopenic septic patients. Furthermore, there was a positive correlation between the two biomarkers and a negative correlation with the platelet count, suggesting a relationship between thrombocytopenia, platelet activation, and increased levels of TLR4.[52] However, a more recent study showed that although there was a tendency toward higher levels of TLR4 expression in the platelets of septic and thrombocytopenic patients, this did not reach statistical significance compared with nonthrombocytopenic septic patients. Remarkably, whereas platelets from normal donors increased their TLR4 expression upon stimulation a similar effect was not observed in septic patients, probably due to the already higher levels of TLR4.[53] In addition, in a very small sample of patients ($n = 7$), Claushuis et al. found that platelets from septic patients showed platelet activation but unaltered TLR4 expression.[23]

Because pre-clinical studies have shown that platelet TLR4 and platelet activation are required for NET formation,[42] we studied the relationship between NETosis and platelet activation in septic and burned patients, that is, two acute inflammatory diseases, one infectious and the other sterile. Both groups of patients presented high levels of DNA-complexes and nucleosomes together with increased expression of both P-selectin and platelet TLR4. However, there was no correlation between NETs and any of the platelet activation markers analyzed.[54] Moreover, we also did not find a correlation between increased platelet TLR4 levels and thrombocytopenia. Although our data indicate that platelet TLR4 does not appear to be involved in NET formation, we cannot rule out that it could have been associated with the small number of patient samples. Additionally, increased platelet TLR4 could have been related to other innate immune responses not addressed in our study, such as the formation of mixed aggregates or bacterial clearance.

Overall, there appears to be a tendency for increased expression of platelet TLR4 during sepsis. Nevertheless, more studies in larger cohorts are necessary to clearly understand the pathophysiologic relevance of this up-regulation.

Finally, evidence is also emerging showing that platelet TLR4 and its interaction with neutrophils is relevant in inflammatory bowel diseases such as Crohn's disease, where differential expression of platelet TLR4 has been found in patients in remission and those with active disease.[55]

6 | INHIBITION OF TLR4

Considering platelets TLR4 relevance in different clinical settings, it is interesting to consider the advances made in modulating its function.

Small molecule inhibitors are synthetic or naturally derived chemical agents widely used in the drug industry. Because of their small size and amphipatic properties, small drugs are capable of crossing the cell membrane and act on specific intracellular targets.[56]

Several small drugs have been shown to have inhibitory effects on the TLR4 pathways. For example, TAK-242 (Resatorvid) blocks the interaction between TLR4 and the adaptor proteins TIRAP and TRAM, leading to a reduction on TLR4 signaling and inflammation.[57] A 2-acetamidopyranoside compound (MW 389) inhibits TNF-α and iNOS RNA increase in LPS-stimulated macrophages in vitro and reduced systemic inflammation in mouse models of endotoxemia and necrotizing enterocolitis.[58] Based on its pre-clinical success, TAK-242 advanced into clinical investigations. However, the results are not promising. In a phase III trial directed to managing severe sepsis, TAK-242 failed to suppress serum cytokine levels (IL-6, IL-8, and TNF-α) compared to the placebo group.[59] A second phase III trial was conducted on the use of TAK-242 for sepsis-induced cardiovascular and respiratory failure, but the trial was terminated due to business decisions and not due to safety or efficacy concerns.[60]

Other developments have been made from drugs previously used for other affections. Angiotensin II receptor blockers (ARBs), and statins have been widely used for the treatment of hypertension and cholesterol, respectively. Recently, it was discovered a novel inhibitory activity of these drugs on TLR2 and TLR4 signaling. Among other examples, Valsartan, from the ARB family, has been shown to decrease proinflammatory cytokine release and infarct size by inhibiting TLR4 signaling,[61] although Candesartan is able to inhibit LPS induced TLR4 activation.[62] Within the statins family, Fluvastatin, Simvastatin, and Atorvastatin have all shown a potent inhibitory effect on the TLR4 pathway and a reduction in vascular inflammation[56]

7 | CONCLUSIONS

Increasing basic and clinical data indicate platelet TLR4 as a molecule that links hemostasis and immunity under infectious and noninfectious conditions. Bearing in mind the great number of circulating platelets, the ability of platelet TLR4 to trigger platelet hemostatic responses, to sense PAMPs or DAMPs and, accordingly, instruct inflammatory leukocytes to react to invading pathogens are among the most interesting and challenging findings in platelet biology in the last decade. However, there are still many questions to be answered. Among them, is platelet TLR4 the molecule linking infection and vascular diseases? Can platelet TLR4 be considered a novel prophylactic and therapeutic target in ACS or a new target to modulate the hemostatic disorder of sepsis? Are the observed sex differences in platelet TLR4 mRNA expression related to TLR4 protein expression? Why does only 40% of the platelet population express TLR4? Do DAMPs and PAMPs modulate TLR4 in megakaryocytes or their precursors? If yes, how does this affect newly formed platelets? The answer to these questions may help us to better understand the role of platelet TLR4 and design better platelet-targeting treatments for patients with vascular or immune diseases.

DISCLOSURES

The author declares no conflicts of interest.

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How to cite this article: Schattner M. Platelet TLR4 at the crossroads of thrombosis and the innate immune response. *J Leukoc Biol*. 2018;1–8. https://doi.org/10.1002/JLB.MR0618-213R