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MULTI-AUTHOR REVIEW

Platelet–bacterial interactions

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Abstract Many bacteria are capable of interacting with platelets and inducing platelet aggregation. This interaction may be a direct interaction between a bacterial surface protein and a platelet receptor or may be an indirect interaction where plasma proteins bind to the bacterial surface and subsequently bind to a platelet receptor. However, these interactions usually do not trigger platelet activation as a secondary co-signal is also required. This is usually due to specific antibody bound to the bacteria interacting with FcγRIIa on the platelet surface. Secreted bacterial products such as gingipains and lipopolysaccharide may also be capable of triggering platelet activation.

Keywords Platelet · Bacteria · Streptococci · Staphylococci · Pathogen

Introduction

The concept of infectious agents playing a role in cardiovascular disease was first suggested by William Osler in 1908. This concept was mostly forgotten about until a series of studies carried out by Clawson and White in the 1970s [1–4]. These studies demonstrated that bacteria were capable of binding to, aggregating and degranulating platelets, thus providing a potential mechanism for the role of infectious agents in cardiovascular disease.

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Platelet function in thrombosis

Platelets are small anucleated cells that originate from the cytoplasm of bone marrow megakaryocytes [5]. Platelets circulate in blood vessels as individual entities that ordinarily do not interact with other platelets or cell types. A series of highly controlled events leading the transition from this resting state to an activated state is rapidly initiated if platelets are exposed to an appropriate stimulus. Disruption of the endothelial cell lining of a blood vessel exposes constituents within the subendothelial matrix, including a variety of adhesive proteins that support initial platelet attachment. Following attachment, platelets undergo intracellular signalling events [6] that lead to simultaneous conformational changes in integrins and mobilisation of intracellular granules [7]. The granules release their contents to the surrounding environment to mediate activation of further platelets and support other aspects of haemostasis. Activated platelets interact with each other through binding of matrix proteins to the activated integrins and form an effective plug at the site of injury that is reinforced by the conversion of fibrinogen to fibrin through the coagulation cascade.

Platelet bacterial interactions

The effects of bacteria on platelets can occur through three general mechanisms. The first is mediated by an increase in inflammatory cytokines due to an immune response to the infection which may lead to platelet activation. Secondly, bacteria may secrete products that activate platelets and, finally, bacteria may bind to platelets. The binding to platelets can be either direct or indirect. Direct binding involves a bacterial surface protein binding to a receptor on

the platelet, while indirect binding is mediated by a protein (usually a plasma protein) that can bind to both the bacteria and the platelet [8]. The effects of inflammation on platelet function are beyond the scope of this review which will focus on direct and indirect interactions of bacteria with platelets as well as secreted bacterial products.

The platelet can respond in two ways to an interaction with bacteria. If bacteria bind strongly to platelets, they can support an adhesive interaction which may be stimulatory resulting in platelet activation, secretion and subsequent aggregation. Thus, platelet adhesion to a bacterium is an indication of the strength of the interaction while aggregation is an indication of the quality of the interaction. The aggregation response to bacteria is different to that of other agonists. It is an all-or-nothing response. There is a threshold concentration of bacteria below which there is no aggregation and above which there is maximum aggregation. There is no intermediate response. Also, unlike other agonists, there is a distinct lag time before aggregation occurs. Increasing bacterial cell concentration shortens this to some extent but never eliminates it. Some bacteria can have a very rapid lag time, as short as 90–120 s while others can take as long as 20 min.

Bacteria display considerable variation in their ability to interact with platelets. We have proposed several different phenotypes for platelet–bacteria interactions [9, 10]. We have identified strains of bacteria that induce platelet aggregation with a short lag time and support direct platelet adhesion [*Streptococcus sanguinis* (133-79) and *Staphylococcus aureus* (Newman)], strains that induce platelet aggregation with a short lag time and support platelet adhesion by an indirect interaction [*Staph. aureus* (Newman) and *Helicobacter pylori* (60190)], strains that induce platelet aggregation with a short lag time and are non-adhesive [*Strep. pneumoniae* (tigr caps 4) and *S. sanguinis* (B10.18)], strains that induce platelet aggregation with a long lag time and support direct platelet adhesion [*Streptococcus gordonii* (DL1) and *S. sanguinis* (M108)], strains of bacteria that induce platelet aggregation with a long lag time and are non-adhesive [*Strep. gordonii* (M99), *S. sanguinis* (NCTC7863) and *S. pneumoniae* (R6x)], strains that do not induce platelet aggregation but do support direct platelet adhesion [*S. gordonii* (Blackburn)], strains that do not induce platelet aggregation but do support platelet adhesion by an indirect interaction [*H. pylori* (J104)] and finally strains that do not induce platelet aggregation or support platelet adhesion [*Strep. sanguinis* (SK96), *S. gordonii* (Channon)] See Table 1.

An interesting aspect of streptococcal induced platelet aggregation, observed by many investigators, is the time course (lag time) to platelet aggregation [9–14]. The average lag time to aggregation following addition of streptococci to platelets is approximately 5–20 min. This is in direct contrast to well-characterised platelet agonists such as adenosine diphosphate (ADP) or thrombin receptor activating peptide (TRAP) which have a lag time of approximately 10 s. Many suggestions have been put forward to explain this lag time, including time taken for the recognition of binding moieties within membrane receptor, binding of plasma proteins [9, 10], including specific antibody [11, 13], fibrinogen or complement and also weak signals generated in the platelet following bacterial binding [15, 16].

There is a growing awareness that platelet–bacterial interactions are more complex than suggested by these interactions. Platelets exist in a dynamic environment where they are exposed to a range of shear stress. Platelets are very sensitive to shear and some platelet–substrate interactions only manifest themselves upon exposure to shear. The best studied shear-dependent interaction is that between platelet GPIb and immobilized von Willebrand factor (vWF) [17]. Under low (venous) shear there is no interaction between platelets and vWF; however, under high (arterial) shear, platelets roll along a vWF-coated surface. Thus, it is important to study platelet–bacteria interactions under a range of shear stress.

Below, we discuss the nature of the interactions of platelets with Streptococci, Staphylococci and *Helicobacter pylori* as these are the best characterised interactions. We will also discuss the role of secreted bacterial products in platelet activation.

Streptococcal platelet interactions

Viridans group Streptococci comprise a large proportion of the commensal bacteria that colonise oral surfaces [18]. These bacteria occasionally enter the blood stream following trauma to the oral cavity [19, 20] and cause infective endocarditis [21] or become implanted in atherosclerotic plaques [22]. Until recently, viridans Streptococci were the most common cause of IE but have now been superseded by *Staph. aureus* [23].

Early studies demonstrated that M protein expressed on the surface of group A Streptococcus isolated from patients with rheumatic fever induced platelet aggregation. This event was primarily mediated in an antibody and complement-dependent manner [24]. Later studies demonstrated that *Strep. pyogenes* and *S. sanguinis* could bind directly to platelets via an unidentified bacterial protein to induce platelet aggregation and support platelet adhesion [12, 25–27] in a reversible and saturable manner [28]. Although these studies were carried out in vitro, the platelet aggregates showed streptococci trapped within, which mimicked signs of macroscopic thrombi found in vivo.

Table 1 Phenotypic summary of platelet bacterial interactions

Direct adhesion	Indirect adhesion	Non-adhesive
Short lag		
Streptococcus sanguinis (133-79) [9]	*Staphylococcus aureus* (Newman)	*Streptococcus pneumoniae* (tigr caps 4)[a]
Staphylococcus aureus (Newman) [11]	*Helicobacter pylori* (60190) [89]	*Streptococcus sanguinis* (B10.18) [9]
Long lag		
Streptococcus gordonii (DL1) [10]	ND	*Streptococcus gordonii* (M99) [10]
Streptococcus sanguinis (M108) [9]		*Streptococcus sanguinis* (NCTC7863) [9]
		Streptococcus pneumoniae (R6x)[a]
Non-aggregating		
Streptococcus gordonii (Blackburn) [10]	*Helicobacter pylori* (J104) [89]	*Streptococcus gordonii* (M5) [10]
		Streptococcus sanguinis (SK96) [9]

Direct adhesion occurs when an interaction between a bacterial adhesion and a platelet membrane receptor occurs. *Indirect adhesion* occurs when a plasma protein binds to a bacterial adhesion which in turn bridges to a platelet membrane receptor. *Non-adhesive* is a strain of bacteria that does not support platelet adhesion in the presence or absence of plasma proteins. *Short lag* time is defined as aggregation that occurs within 8 min from addition of bacteria. *Long lag* time is defined as aggregation that occurs greater than 8 min but less than 20 min. *Non-aggregating* is defined as a strain of bacteria that does not induce platelet aggregation

[a] Unpublished observations (Kerrigan, Kadioglu, Jenkinson, Cox)

Several streptococcal surface components have now been identified and are being investigated for their role in binding to and activating platelets. Platelet-associated activating protein (PAAP) was first identified in 1990. PAAP is synthesised as a 115-kd *N*-asparagyl-linked glycoprotein [29–32]. The protein backbone contains a collagen-like epitope that induces platelet aggregation. PAAP is strongly suggested to be a cell surface heat shock-inducible chaperone, with consensus glycosylation and myristoylation sites based upon a motif mapping comparison [31]. Therefore, PAAP expression may be environmentally regulated during infection in response to heat shock (fever) or collagen (exposed on damaged heart valves), enabling the bacteria to move more efficiently to recruit platelets. PAAP interacts with a signal transducing receptor, inducing platelet activation and aggregation. However, the identity of the platelet receptor for PAAP is still unclear. Gong and colleagues reported that PAAP interacts with a platelet membrane protein of 175 and 230 kDa to mediate platelet binding and aggregation [33]. Recent reports suggest that the role of PAAP in inducing platelet aggregation may be donor specific [34].

Kerrigan et al. identified three different phenotypes for streptococcal induced platelet aggregation. Type I have a short lag time to platelet aggregation, Type II have a long lag time to platelet aggregation and type III do not induce platelet aggregation at all [9]. Platelet aggregation induced by type I strains is mediated by a direct interaction between *Strep. sanguinis* and platelet glycoprotein Ibα (GPIbα), the von Willebrand factor (vWF) receptor. This interaction was localised to the N-terminal of GPIbα between residues 1 and 225 [9]. Furthermore, platelets from patients with Bernard Soulier Syndrome, who fail the express GPIbα, do not aggregate in response to *S. sanguinis*. The *S. sanguinis* protein that interacts with platelet GPIbα is a serine-rich glycoprotein called SrpA [14]. Deletion of SrpA does not abolish platelet aggregation but does prolong the lag time, suggesting other interactions occur. Type II strains have a long lag time and may require antibody binding and complement assembly [13, 35]. Early studies suggested that IgG was not required for type II *S. sanguinis* induced platelet aggregation as aggregation occurred in a plasma-free system with only fibrinogen present [9]. The problem with these experiments is that commercial fibrinogen often contains small amounts of contaminating IgG, thus providing sufficient IgG to support an antibody mediated response. More recently McNicol and colleagues demonstrated that depletion of *S. sanguinis* specific antibodies from plasma significantly inhibited platelet aggregation [36]. Moreover, rapid phosphorylation of FcγRIIa occurred following *S. sanguinis* binding [15].

Initial reports suggested that another oral pathogen, *Strep. gordonii* could not induce platelet aggregation [37]. However, it is now well established that *S. gordonii* can adhere to and induce platelet aggregation. GspB is a 286-kDa surface anchored protein which interacts with platelets through the recognition of specific sialic acid residues found on GPIbα [38–40]. The primary role of GspB is to support bacterial adhesion to the tooth pellicle [40, 41]. GspB is glycosylated in the cytoplasm and is then transported to the cell surface via an accessory system compromising of the SecA2 and SecY2 proteins [40]. GspB is similar to that of an expanding family of Gram positive bacterial cell surface proteins that includes *S. gordonii* Hsa [42] and *S. parasanguinis* Fap1 [43]. Hsa is a 203-kDa sialic acid-binding protein that plays an

essential role in binding to and inducing platelet aggregation [10, 41, 44]. Hsa binds specifically to the N-linked sialic acid residues on GPIbα, and GPIIb/IIIa [45], whereas GspB binds to O-linked sialic acids as well as the membrane proximal mucin-rich core of GPIbα [40].

Most species of oral streptococci express high molecular weight cell wall associated antigen I/II family polypeptides, designated SspA (172 kDa) and SspB (164 kDa) in *Strep. gordonii* [46]. These polypeptide adhesins recognise multiple ligands including salivary agglutinin glycoprotein (gp-340) [47], collagen type I [48], β1 integrins [46], and other oral micro-organisms such as *Porphyromonas gingivalis*, *Candida albicans* and *Actinomyces naeslundii* [49–52]. Deletion of SspA and SspB from *S. gordonii* does not affect platelet adhesion, but extends the lag time to platelet aggregation. Deletion of SspA and SspB and Hsa from *S. gordonii* reduces platelet adhesion by 50% but abolishes platelet aggregation [10]. These results suggest that *S. gordonii*-induced platelet aggregation and adhesion is a multifactorial event mediated by several surface proteins.

Strep. mitis has been shown to bind to platelets via surface proteins PblA and PblB. Upon binding platelets, these proteins do not generate an intracellular signal leading to platelet activation [53, 54]. *Strep. pyogenes* and *S. pneumoniae* both induce platelet aggregation in an antibody-dependent manner [27, 55]. *S. pyogenes* M1 protein has been shown to bind fibrinogen which in turn interacts with GPIIb/IIIa [56]. The presence of anti-M1 antibody in this complex can interact with FcγRIIa and induce platelet aggregation in a similar manner to *Staph. aureus* ClfA and FnbpA (see below).

Under fluid shear conditions, platelets interacted with immobilised *Strep. sanguinis* or *S. gordonii* with a typical rolling behaviour followed by firm adhesion [10, 14]. This rolling behaviour followed by firm adhesion is typical of platelet interactions with subendothelial matrix proteins at sites of vessel injury [17]. It occurs as a result of platelet GPIbα binding to endothelium-bound vWF. This interaction occurs under conditions of high shear, but does not occur under conditions where low shear is experienced. In contrast to this, platelets interact with *S. sanguinis* or *S. gordonii* under low-shear conditions but not under high-shear conditions. This suggests that Hsa and SrpA must exist in a suitable conformation for direct interaction with GPIbα under low-shear conditions. Deletion of Hsa from *S. gordonii* or SrpA from *S. sanguinis* ablated platelet interactions under all shear conditions, suggesting that this family of serine-rich glycoproteins are critical for firm adhesion [10, 14]. It also suggests that this event is most likely mediated by an interaction with platelet GPIbα. Thrombus formation by *S. pyogens* has also been studied under high shear conditions where M protein, specific IgG and fibrinogen are required for rapid thrombus formation [57].

Staphylococcal platelet interactions

A comprehensive study by Siegel and Cohen demonstrated that a crude extract from Staphylococcus led to distinctive degenerative changes in the platelet as evidenced by microscopic examination and loss of single platelets as evidenced by turbidimetric aggregometry [58]. Following this, Bernheimer and Schwartz identified the crude extract as being α-toxin with lytic properties and ruled out platelet aggregation as a cause in decrease in single platelet suspension [59, 60]. Subsequent studies demonstrated that Staphylococcal surface protein A acts as a receptor for specific anti-staphylococcal antibodies which in turn bind FcγRIIa on platelets. This event led to the release reaction and GPIIb/IIIa-dependent platelet aggregation [61]. More recent studies demonstrated that *Staph. aureus* could adhere to platelets via a fibrinogen/fibrin bridge [62]. A mutant of *S. aureus* lacking clumping factor A (ClfA) failed to adhere to platelets, suggesting that ClfA binds fibrinogen which in turn binds the platelet fibrinogen receptor, GPIIb/IIIa [63]. It was also suggested that ClfA could bind to an as yet unidentified protein of 118 kDa on the platelet surface. Deletion of one or more genes or heterologous expression in a surrogate host has identified several proteins on *S. aureus* that bind to platelets and induce platelet aggregation [11].

As part of their survival, bacteria often express a different profile of proteins on their surface at different stages of growth. ClfA is the dominant proaggregatory surface protein [64] in *Staph. aureus* cells grown to stationary phase whereas fibronectin binding proteins (FnBP) are the most dominant proaggregatory surface protein [65] in *S. aureus* cells grown to exponential phase. This correlates with the regulated expression of FnBPA and FnBPB which are expressed in exponential phase of growth but not at stationary phase of growth [66] and ClfA which is weakly expressed at exponential phase of growth and strongly expressed at a stationary phase of growth [64].

A plasma-free system was used to determine the factors necessary for *Staph. aureus*-induced platelet aggregation [64]. Addition of fibrinogen and ClfA-specific immunoglobulin to the plasma-free system led to *S. aureus*-induced platelet aggregation. Even though resting GPIIb/IIIa has little or no affinity for soluble fibrinogen, it can still bind fibrinogen bound to bacteria; however, this is not enough to trigger activation. To trigger full platelet activation, both fibrinogen and specific immunoglobulin must bind to the A domain on ClfA. There are two distinct sites on ClfA that allows fibrinogen and IgG binding at the same time [64]. Once bound, fibrinogen molecules can engage resting GPIIb/IIIa, aided by bound ClfA-specific immunoglobulin, which encourages the clustering of Fc receptor, FcγRIIa. This triggers activation of signal transduction leading to

conformational change in GPIIb/IIIa and aggregation of platelets.

As all the previous studies have been carried out under static or non-physiological stirring conditions, it is difficult to relate these studies to the disease process as cells in the vasculature experience a wide range of shear rates. Studies using a cone and plate viscometer have shown that protein A, ClfA, SdrC, SdrD and SdrE are important in thrombus formation [67–69]. However, extremely high shear rates were used in these rheological studies. When platelets in whole blood were perfused over immobilised *Staph. aureus* under shear conditions equivalent to arterial pressure, very strong adhesion occurred followed by rapid aggregate formation [70] using a parallel flow chamber. Deletion of ClfA from *S. aureus*, abolished adhesion and aggregate formation under all shear rates investigated. Using a plasma-free system, fibrinogen led to single platelet adhesion but not aggregate formation. Specific immunoglobulin failed to have any effect on either platelet adhesion or aggregation. However, addition of fibrinogen and specific immunoglobulin to the plasma-free system led to platelet adhesion followed by aggregate formation [70], thus highlighting the importance of fibrinogen and IgG in aggregate formation induced by *S. aureus*. No interaction was seen under low shear conditions using a parallel flow chamber.

Deletion of the fibrinogen binding domain in ClfA (ClfA-PY) led to the discovery of a second pathway that *Staph. aureus* uses to induce platelet aggregation [64]. ClfA-PY induced platelet aggregation after a long lag time (between 8 and 20 min). However, to trigger platelet activation, specific immunoglobulin must bind to the A domain on ClfA which in turn will bind FcγRIIa on the platelet. This is not enough to trigger platelet activation, and therefore complement must assemble on the *S. aureus* surface and then bind to unidentified complement receptors on the platelet. Both complement and specific immunoglobulin are required for activation to occur [64].

Fibronectin binding proteins contain a specific immunoglobulin binding domain (A domain) and a fibronectin binding domain (BCD). The FnBPA A domain is similar in structure and function to that of the ClfA A domain. FnBPA possesses two different but related mechanisms of engaging and activating platelets [65]. In the first mechanism, fibrinogen can bind to the A domain which cross-links to GPIIb/IIIa, and specific immunoglobulin must cross-link to FcγRIIa to trigger platelet activation and aggregation [65]. In the second mechanism, the fibronectin binding domain, BCD, can independently activate platelets. Fibronectin can bind to *Staph. aureus* via the FnBPA BCD domain by the tandem β-zipper mechanism [71–73] and also to platelet GPIIb/IIIa through the common integrin recognition motif RGD [65]. The signal to trigger platelet activation/aggregation is complete when specific immunoglobulin binds the A domain of FnBPA and cross-links to platelet FcγRIIa.

Clumping factor B is a fibrinogen-binding protein which is highly expressed in the exponential phase of growth and shares structural homology with ClfA [74]. ClfB can also bind fibrinogen and specific immunoglobulin to trigger platelet aggregation similar to ClfA and FnBP. A non-fibrinogen binding ClfB mutant triggered platelet aggregation following complement assembly which also required specific antibody [75]. Staphylococcal protein A (SpA) has been previously shown to bind platelet directly to the complement receptor gC1qR/p33 [76]. Typically found intracellularly, this receptor is only brought to the surface of platelets following activation.

Recent work has demonstrated that all five domains of SpA (A-E) can bind to the A1 domain of von Willebrand factor with high affinity (low nM range) [77, 78]. The von Willebrand factor receptor on platelets is GPIbα therefore it is possible that *Staph. aureus* SpA binding vWf leads to agglutination or cross-linking of platelets rather than true aggregation. Furthermore, Pawar and colleagues demonstrated a key role for SpA in mediating platelet activation at high shear rates [67]. A monoclonal antibody directed against vWF partially inhibited platelet activation and an antibody directed against the platelet vWF receptor, GPIbα, also partially inhibited, highlighting the importance of the interaction between vWf and GPIbα under high shear conditions [67].

SraP is a member of the serine-rich highly glycosylated family of proteins. It has high homology to *Strep. gordonii* GspB/Hsa and *S. sanguinis* SrpA. The interaction of GspB/Hsa and SrpA with sialic acid residues on platelet GPIbα is well characterised. In a rabbit model of endocarditis SraP was shown to promote *Staph. aureus* binding to platelets and increase virulence, though not through GPIbα [79].

Helicobacter pylori platelet interactions

Helicobacter pylori are Gram negative bacteria that play a role in the pathogenesis of peptic ulcer disease, gastric carcinoma and primary B cell gastric lymphoma [80]. Some studies have shown the formation of platelet aggregates in *H. pylori*-infected patients [81] which may explain the association between *H. pylori* and cardiovascular disease, such as myocardial infarction [82–84] and stroke [85, 86] although others have failed to show any link [87]. Clinical strains of *H. pylori* have been shown to induce platelet aggregation in vitro by binding plasma vWF which in turn binds to platelet GPIbα triggering an activating response [88, 89]. Antibodies against vWF or GPIbα prevented *H. pylori*-induced platelet aggregation.

Furthermore, patients with Bernard Soulier Syndrome (who lack expression of GPIbα) fail to aggregate in response to *H. pylori* [89]. *H. pylori*-induced platelet activation was dependent on binding plasma vWF and specific immunoglobulin and then bridging to GPIbα and FcγRIIa, respectively, to trigger platelet activation. This interaction differs from *Strep. sanguinis*-induced platelet aggregation which also binds GPIbα, as it binds directly to GPIbα directly independent of vWF.

Secreted products

Lipopolysaccharide (LPS) is an essential component of Gram-negative bacteria cell wall that is shed into plasma. LPS interacts with Toll-like receptors (TLR) on immune cells and this reaction is a key component of the immune response to infection [90]. The concept that platelets contain TLRs and can aggregate in response to LPS is controversial. Initially LPS was shown to induce platelet secretion [91, 92], but LPS was subsequently shown not to bind to platelets nor to induce aggregation, and neither CD14 (a key component of TLR4 signalling complex) nor TLR4 were identified on the platelet surface [93]. However, this was followed by a report that TLR1 and TLR6 were present on platelets [94], and subsequently TLR2, TLR4 and TLR9 were found on the platelet surface [95–97]. However, the functional relevance of these TLRs is unclear as the TLR4 agonist LPS was unable to induce platelet aggregation or even enhance ADP-induced platelet aggregation [98]. However, *Escherichia coli* O157 LPS was shown to bind to and mediate activation of platelets in a TLR4-dependent manner [99] and to enhance platelet secretion of cytokines [100]. Chicken thrombocytes were also shown to express TLR4 receptor and to become activated by LPS [101]. There seems to be variation in the ability of different types of LPS to bind to platelets, and LPS from *E. coli* O157 appears to be the most potent [99].

So, while it appears that TLRs are present at low levels on platelets, it is not clear if they are functional receptors and certainly there is no consensus on the ability of TLR4 to mediate platelet aggregation by LPS. However, there is evidence to suggest that the actions of LPS on platelet activation may be indirect. LPS was found to bind to TLR4 on platelets but not induce aggregation. However, these LPS bound platelets had increased affinity for neutrophils and only LPS-treated platelets were capable of inducing neutrophil activation [102]. LPS was also shown to induce thrombocytopenia in mice that was neutrophil-dependent [96]. Thus, LPS binds to platelet TLR4 but does not generate an activation signal, at least not one than leads to platelet aggregation. However, it does prime the platelets allowing them to bind to and activate neutrophils.

Lipoteichoic acid (LTA) is secreted by Gram-positive bacteria and is a TLR2 agonist. LTA was shown to bind to platelets and to inhibit platelet aggregation by collagen [103] as well as to support platelet adhesion to *Strep. epidermidis* [104]. It was suggested that the anti-platelet effect of LTA was due to conformational changes in the membrane [105] and an increase in cAMP levels [106]. The TLR2 agonist, Pam_3CSK_4, was unable to induce platelet aggregation or even enhance ADP-induced platelet aggregation [98]. Thus, there is little evidence to suggest that TLR2 receptor on the platelet surface can mediate platelet activation.

Other than the cell wall components LPS and LTA, bacteria secrete substances that can induce platelet activation. *Porphyromonas gingivalis* secretes gingipains which are proteases that can directly activate platelets. This is due to activation of protease-activated receptors on the platelet surface [107, 108]. Shiga-like toxin (verotoxin) secreted by *E. coli* was shown to induce platelet aggregation [109] and both Shiga and Shiga-like toxins were shown to bind to glycosphingolipid receptors on the platelet surface [110]. However, Shiga toxin was subsequently shown to have no effects on platelet aggregation [111] and to only bind to activated platelets [112], although others showed that exposure to Shiga toxin did lead to platelet activation [113]. One explanation for these contradictory results is that in vivo the actions of Shiga toxins are complex and many of its actions on platelets are indirect, being mediated through effects on other cells such as endothelial cells [114] and monocytes [115]. α-toxin is a pore-forming toxin produced by *Staph. aureus* which is responsible for haemolysis. It also leads to platelet activation [116] leading to the assembly of the prothrombinase complex on the platelet surface [117].

FcγRIIa

While all these bacteria have different mechanisms for interacting with platelets there is a common feature to platelet aggregation induced by all these bacteria. Excluding aggregation that is induced by the secreted products, bacteria-induced platelet aggregation is generally inhibited by antibodies to FcγRIIa. FcγRIIa is the platelet IgG receptor and is a member of a family of Fc receptors which mediate the cellular responses to the Fc portion of antibodies [118]. Blockade of FcγRIIa has been shown to prevent aggregation by *Strep. sanguinis* [9, 13], *S. gordonii* (unpublished data), *H. pylori* [89], *S. pyogenes* [56] and *Staph. aureus* (both direct activation and complement-dependent activation) [64, 65, 75]. In all cases, antibody was required for an aggregation response. However, FcyRIIa engagement by antibody was insufficient to induce

aggregation and in each case engagement of another receptor such as GPIbα [9, 89], GPIIb/IIIa [64, 65] or complement receptor [35, 75] was also required. Thus, FcγRIIa requires cross-linking with the formation of either homodimers as occurs with agglutinated IgG [119, 120] or the formation of heterodimers with GPIIb/IIIa or GPIbα for the necessary signaling to occur. In fact, there is evidence of co-localisation of FcγRIIa with both GPIbα [121] and GPIIb/IIIa [122]. Thus, FcγRIIa may be an ideal drug target due to its essential role in platelet activation by bacteria.

Clinical implications

It is clear that platelets are part of the innate immune system and play a role in the host response to infection. However, under certain circumstances, the platelet response to infection may be a significant part of the problem. Activation of platelets by bacteria can lead to three specific problems. Activation of platelets in a localised manner can lead to thrombus formation while a more systemic activation can lead to platelet consumption. Finally, activated platelets secrete many cytokines and other mediators that can trigger pathological processes.

Infective endocarditis is a typical example of a thrombotic complication of bacterial infection. It is due to infection of the heart valve by bacteria, typically *Staph. aureus* or an oral Streptococcus [123]. While the precise sequence of events is not clear, a bacteria–platelet thrombus forms on the valve which can either lead to valve failure or the formation of a septic embolus. Treatment requires antibiotic therapy and often valve replacement surgery.

Systemic bacterial infection such as occurs during septicemia leads to thrombocytopenia and bleeding complications. This is a serious disease with poor outcome [124]. Platelet activation during sepsis [125] leads to platelet sequestration, thrombocytopenia and bleeding complications. The extent of thrombocytopenia is related to outcome [126, 127]. Haemolytic uremic syndrome is due to the formation of microthrombi in the glomerular capillaries usually as a result of an *E. coli* infection. This results in reduced glomerular filtration and subsequently to renal failure. Thrombocytopenia also occurs usually due to damage to the platelets as they pass through the stenosed vessels and possibly due to actions of Shiga toxin on the platelets [128].

When activated, platelets secrete their granule contents which contain at least 300 different proteins including cytokines and vascular active factors [129, 130]. These cytokines play a key role in the pathogenesis of atherosclerosis [129, 131–134] and may also explain the association between infection and cardiovascular disease. As well as causing thrombocytopenia, sepsis also leads to shock due to endothelial inflammation and subsequent vascular leakage. Activated platelets play a key role in mediating the endothelial damage [125, 135, 136].

With the growing incidence of infection with antibiotic-resistant bacteria such as MRSA, the management of the patient with an infection is becoming more difficult. In diseases such as infective endocarditis and sepsis, platelets are not innocent bystanders but active participants in the disease process. Targeting the platelet may help stabilise the patient and reduce the impact of some of the serious consequences of these diseases such as bleeding, shock and thrombosis. FcγRIIa may be the ideal drug target. Unlike other anti-platelet agents, inhibitors of FcγRIIa do not affect the platelet response to other agonists and thus does not compromise platelet function.

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