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CITATION

Cox, Dermot; Kerrigan, Steven W.; Watson, Steve (2011): Platelets and the innate immune system: Mechanisms of bacterial-induced platelet activation.. Royal College of Surgeons in Ireland. Journal contribution. https://hdl.handle.net/10779/rcsi.10782758.v1

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Platelets and the innate immune system: Mechanisms of bacterial-induced platelet activation

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Summary.

It has become clear that platelets are not simply cell fragments that plug the leak in a damaged blood vessel; they are, in fact, also key components in the innate immune system, which is supported by the presence of Toll-like receptors (TLRs) on platelets. As the cells that respond first to a site of injury, they are well placed to direct the immune response to deal with any resulting exposure to pathogens. The response is triggered by bacteria binding to platelets, which usually triggers platelet activation and the secretion of antimicrobial peptides. The main platelet receptors that mediate these interactions are glycoprotein (GP)IIb–IIIa,GPIbα,FcγRIIa, complement receptors, and TLRs. This process may involve direct interactions between bacterial proteins and the receptors, or can be mediated by plasma proteins such as fibrinogen, von Willebrand factor, complement, and IgG. Here, we review the variety of interactions between platelets and bacteria, and look at the potential for inhibiting these interactions in diseases such as infective endocarditis and sepsis.

Introduction

Haemostasis is a critical process that acts to seal breaches in the vascular system. This serves two functions: prevention of further blood loss and denial of access for pathogens to the vascular system. Platelets are key mediators of this response and act to stop the leak and facilitate wound healing. In addition, platelets play a key role in preventing infection. When activated, platelets secrete the contents of their granules which are known to contain over 300 proteins [1] as well as bioactive molecules such as ADP and serotonin. ADP acts to recruit more platelets into the growing thrombus while serotonin causes vasoconstriction to reduce blood loss. Secreted cytokines and chemokines recruit leucocytes to deal with any potential infection and secreted anti-microbial peptides act to kill pathogens.

While thrombus formation at the site of a wound prevents blood loss, it can also occur in a diseased vessel such as a coronary or cerebral artery causing

a potentially fatal myocardial infarction (MI) or stroke. Equally, activation of platelets by pathogens at locations other than a wound can lead to serious consequences such as infective endocarditis (IE) or disseminated intravascular coagulation (DIC). However, while therapies have been developed to prevent thrombosis in stroke and MI, it is essential to develop therapies to prevent pathogen-induced platelet activation which will in all probability be different from existing anti-platelet agents.

The activation of platelets leads to secretion of anti-microbial peptides, although many bacteria have become resistant to these peptides [2]. Bacteria have also developed the ability to interact with platelets without inducing platelet activation which allows them to adhere to surfaces coated with platelets such as a damaged cardiac valve. This ability to bind to platelets without activating them or to be resistant to their anti-microbial actions enables bacteria to survive in the circulation either surrounded by or phagocytosed by platelets and invisible to leucocytes.

Infection and thrombosis

As platelets are usually the first cells to respond to a wound they have an important role in regulating the host response to infection which is by platelet activation by bacteria [3, 4]. However, this process can contribute to diseases such as infective endocarditis, a serious infection of the heart valves usually due to infection with either staphylococci (e.g. *S. aureus*) or streptococci (e.g. *S. sanguinis* or *S. gordonii*). IE is due to the formation of a bacteria-platelet thrombus on one of the heart valves which, as it grows, either leads to valve failure requiring valve replacement or to the formation of a septic embolus which can cause a stroke, heart attack or pulmonary embolism. The major risk factors for IE are dental disease or manipulation and intravenous drug abuse which allow entry of oral streptococci and *S. aureus* respectively into the blood stream [5].

Another thrombotic disease associated with infection is septicemia [6]. Patients with septicemia typically develop DIC characterized by microthrombi

formation which can lead to blockage of the microvasculature and organ damage. Thrombus formation can also lead to consumption of coagulation factors and platelets placing the patient at risk of a bleeding event [7]. Thrombocytopenia due to platelet activation in sepsis is a common occurrence and its extent is related to outcome [8].

The studies of bacterial interactions with platelets have primarily been confined to Gram-positive bacteria, especially staphylococci (*S. aureus* and *S. epidermidis*) and streptococci (*S. sanguinis* and *S. gordonii*), although the interaction with Gram-negative *Helicobacter pylori* has also been characterized [4].

Mechanisms of interaction

There are three basic mechanisms that are used to mediate the interaction between pathogens and platelets: 1) Binding to bacteria of a plasma protein that is a ligand for a platelet receptor 2) Direct bacterial binding to a platelet receptor 3) Secretion of bacterial products i.e. toxins that interact with platelets. This presence of multiple mechanisms makes it difficult to identify the roles of the different proteins (both bacterial and platelet) which is further complicated by interactions that are not only species-specific but strain-specific as well. Some interactions lead to platelet activation while others have no effect on the platelet. These non-activating interactions are usually of high affinity and probably play a role in supporting platelet adhesion under the shear conditions found in the circulation. Typically bacterial proteins that mediate adhesion are distinct from those that mediate aggregation. Thus, bacteria can support platelet adhesion and/or trigger platelet activation.

Bacterial-induced platelet aggregation is often uniquely characterised by a distinct delay known as the lag time (Fig. 1). When a soluble agonist such as ADP is added to a platelet suspension the aggregation response happens within a few seconds. When bacteria are added to a suspension of platelets, there is a delay in the aggregation response that is concentration-dependent.

Increasing the bacteria concentration shortens this lag time but never abolishes it. Bacteria such as *S. aureus* induce platelet aggregation with a lag time of around 2-3 minutes. However, a complement-dependent strain such as *S. sanguinis* NCTC 7863 usually takes between 10-15 minutes to induce aggregation. Unlike soluble agonists, bacterial-induced platelet aggregation is all-or-nothing.

Platelet receptors

While bacteria utilize many different proteins to interact with platelets, there are a limited number of platelet receptors that mediate adhesion and / or activation, notably GPIIb/IIIa, GPIb and FcγRIIa (Fig. 2 & 3). This limited number of platelet receptors makes it possible to realistically develop anti-platelet agents that target a wide-range of bacteria-platelet interactions.

GPIIb/IIIa.

GPIIb/IIIa is the most abundant platelet surface membrane glycoprotein and its expression is specific to platelets and megakaryocytes. GPIIb/IIIa is a member of the integrin family of heterodimeric receptors that mediate cell adhesion and signalling. Resting platelets contain approximately 80,000 surface copies with additional pools of GPIIb/IIIa in the membranes of α-granules and the open canicular system. Upon platelet activation, surface expression can increase as much as 50%. As the platelet fibrinogen receptor GPIIb/IIIa mediates cross-linking of platelets by fibrinogen which is responsible for aggregate formation [9].

Fibrinogen-binding proteins

Staphylococci have a family of surface receptors that are members of the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM) family of proteins, often characterized by the presence of a domain rich in serine-aspartate repeats (Sdr) [10]. Examples of MSCRAMMs include *S. aureus* clumping factors (Clf) A [11] and B [12] and fibronectin-binding proteins (FnBP) A and B [13]; *S. lugdunensis* Fbl [14]; *S. epidermidis*

Sdr G [15]. Most of the MSCRAMMs bind plasma proteins such as fibrinogen or fibronectin [16].

While fibrinogen-binding MSCRAMMs are related proteins, they bind to different domains in fibrinogen. Both Clf A and Fbl bind to the C-terminal region of the fibrinogen γ-chain [17] as do the non-Sdr proteins Fnbp A and B. Clf B binds to the C-terminus of Aα chain [18] and Sdr G to the Bβ-chain [19]. Fibrinogen-bound bacteria mediate platelet activation in a similar way to other fibrinogen-coated surfaces. As its name implies, Fnbp also binds fibronectin and this can also bind to GPIIb/IIIa [20]. In all cases, the MSCRAMM-bound fibrinogen/fibronectin can interact with GPIIb/IIIa generating an outside in signal capable of triggering platelet activation.

Streptococci also contain fibrinogen-binding proteins such as *S. pyogenes* M1 protein which triggers platelet aggregation [21]. *S. mitis* lysin binds to the α and β subunits of the fibrinogen D fragment, although it is not known if this induces platelet aggregation [22]. While both proteins are shed/secreted from the bacteria, lysin probably remains associated with the bacterial surface due to its choline-binding properties.

Direct binding to GPIIb/IIIa

More recently, reports have demonstrated that some bacteria express proteins that can directly bind to GPIIb/IIIa in the absence of a bridging molecule. *S. epidermidis* Sdr G can bind directly to GPIIb/IIIa and can also crosslink GPIIb/IIIa and FcγRIIa [15]. More recently, a heme-binding protein on *S. aureus*, IsdB has been shown to support platelet adhesion and induce platelet aggregation through a direct interaction with GPIIb/IIIa [23]. *S. gordonii* also expresses Platelet Adhesion Binding protein A (PadA) a novel high molecular weight protein which directly binds to GPIIb/IIIa and is critical for supporting platelet adhesion but not platelet aggregation [24]. The site of interaction between IsdB or PadA and GPIIb/IIIa has not yet been mapped, although it is noteworthy that preincubating platelets with the peptide mimetic, RGD, completely abolishes adhesion.

GPIbα

GPIbα is a member of the leucine-rich repeat family of proteins which is exclusively expressed on platelets and megakaryocytes. It can bind several different ligands but its crucial role in primary haemostasis relies on its ability to interact with von Willebrand factor (vWF). GPIbα exists in a complex with GPIbβ, GPIX and GPV in a ratio of 2:2:2:1. Platelets express roughly 25,000 copies of GPIbα which mediates both platelet tethering to surface exposed VWf and supports platelet activation under high shear conditions [25].

Several species of streptococci have been shown to directly interact with GPIbα, mediated by a family of serine-rich glycoproteins. This family includes the *S. sanguinis* protein SrpA [26] and *S. gordonii* GspB [27, 28, 29] and Hsa [30], which are all structurally related. These are large, highly-glycosylated, serine-rich proteins that bind sialic acid residues on host receptors. GspB predominantly binds O-linked sialic acid residues while Hsa predominantly binds to N-linked sialic acid residues [29]. The interactions with GPIbα trigger platelet aggregation and support platelet adhesion. *S. aureus* expresses SraP which is a homologue of GspB and supports platelet adhesion [31], possibly through GPIbα.

Just as some bacterial proteins can bind fibrinogen, there are also vWf-binding proteins on bacteria. *S. aureus* protein A has been shown to bind vWf which in turn can interact with GPIbα [32]. *H. pylori* has also been shown to bind plasma vWf through an unknown protein, which in turn enables it to interact with GPIbα and trigger platelet aggregation [33]. Unlike soluble or immobilised vWf, bacteria-bound vWf can interact with GPIbα in the absence of high shear. However, it is not clear if the interaction with vWf is simply an adhesive interaction mediating the binding of bacteria to platelets thereby facilitating an interaction with an activating receptor or whether it also plays a role in platelet activation. Certainly the protein A-mediated interaction does not lead to platelet activation while the *H. pylori*-mediated interaction leads to platelet activation although only through engagement of FcγRIIa.

Toll-like receptors.

Toll-like receptors (TLR) are a family of receptors in the innate immune system that mediate the host response to infection. These receptors recognise conserved pathogen-associated molecular patterns (PAMP) found on different classes of infectious agents [34]. To date at least 11 TLRs have been described in various immune and non-immune cells. Recently platelets have been reported to express TLR 2, 4 and 9 with very weak expression of 1, 6 and 8 reinforcing their role as primitive immune cells in host defense [35, 36]. The discovery of TLRs on platelets led to a search for their role in platelet function with most studies focusing on TLR 2 and 4.

The ligand for TLR 4 is lipopolysaccharide (LPS) from Gram-negative bacteria [37]. Some studies have shown that LPS can induce platelet aggregation [38, 39, 40, 41] while others have shown no effect [42] or even inhibition of platelet aggregation [43]. Exposure to LPS has also been shown to reduce platelet adhesion to fibrinogen in a calcium-dependent process [44]. More recently, it has been shown that rather than induce platelet aggregation LPS leads to enhanced formation of neutrophil-platelet complexes leading to the formation of neutrophil extracellular traps [45] and that LPS-induced thrombocytopenia in mice is neutrophil-dependent [46] which is due in part to increased phagocytosis [47]. LPS also induced tissue factor expression on endothelial cells and monocytes which in turn serves as a binding site for platelet GPIIb/IIIa [48]. In addition, LPS stimulated the release of sCD40L from platelets, which is widely regarded as a predictive indicator of cardiovascular events such as stroke or MI [49], as well as TNF release [36]. Soluble CD40L release is significantly reduced using a blocking monoclonal antibody against TLR4 [50]. *E. coli* O157 LPS has been shown to bind to platelet TLR4 leading to activation [51] although other studies have failed to show any effect of LPS on platelet aggregation [42].

The natural ligand for TLR 2 is lipoteichoic acid [52] and this has been shown to have mixed effects on platelet aggregation. It has been shown to inhibit platelet aggregation and to support platelet adhesion to *S. epidermidis* [53].

Studies using Pam_3CSK_4, a synthetic TLR2 agonist, have shown no effect on platelet aggregation at concentrations that activate TLR2 in other cell types [42], although it did induce aggregation and formation of platelet-neutrophil aggregates at a ten fold higher concentration in wild-type but not TLR2-deficient mice [54]. More recently it was shown to induce platelet aggregation and secretion in an ADP receptor-dependent manner [55]. *S. pneumoniae* was shown to induce platelet aggregation in a TLR2-dependent manner and also generated an intracellular signal that triggered dense granule release and activated the phosphoinositide-3-kinase (PI3-kinase)-RAP1 pathway [56]. However, *S. aureus*-derived lipoteichoic acid has been shown to inhibit platelet aggregation [57].

While the molecular basis of these effects is still unclear it appears that the primary effect of TLR-mediated activation of platelets is the secretion of immunomodulatory agents and the activation of other cells such as neutrophils and endothelial cells rather than the formation of a thrombus. In this context platelets are acting as components of the innate immune system [58] rather than components of haemostasis as they act to detect the presence of infectious agents and coordinate the response to the pathogen.

Complement receptors

When bacteria enter the blood they frequently trigger complement generation either in an antibody-dependent manner or an antibody-independent manner (alternative pathway) [59]. Complement-coated bacteria have been shown to be capable of inducing platelet aggregation. Some strains of *S. sanguinis* have been shown to induce platelet aggregation in a process that involves complement but also requires antibody binding [60, 61]. Human gC1q-R is a multi-ligand binding protein for the first component of complement, C1q [62]. Low levels of gC1q-R are expressed on platelets under resting conditions, however upon activation the receptor number increases [63], thus possibly serving as a receptor for complement-coated *S. sanguinis*. *S. aureus* Clf A and B can induce platelet aggregation in a complement- and antibody-dependent process [64, 65]. In all cases complement-mediated aggregation is

FcγRIIa-dependent. It would appear to be dependent on the presence of an unidentified complement receptor on platelets as well.

FcγRIIa

The Fc portion of antibodies mediates its effects through a family of receptors known as Fc receptors. Each antibody type has a sub-family of Fc receptors with IgG interacting with the FcγR sub-family. FcγRIIa is the most widely distributed Fcγ receptor in nature. It is predominantly expressed on neutrophils, monocytes, macrophages and platelets. FcγRIIa is a low affinity IgG receptor with approximately 2000-3000 copies per platelet. It consists of a single transmembrane domain, a C-terminal that contains the binding site for IgG and a cytoplasmic domain. The cytoplasmic domain contains two YXXL sequences separated by twelve amino acids that together constitute an immunoreceptor tyrosine-activation motif (ITAM) [66].

Evidence suggests that FcγRIIa plays a critical role in bacterial-induced platelet aggregation [33, 61, 65, 67, 68]. The role of FcγRIIa is not just as an IgG receptor but it also plays an important role in platelet function. FcγRIIa enhances GPIIb/IIIa-mediated platelet spreading on fibrinogen in an IgG-independent manner [69]. It has also been shown to be co-localized with GPIbα and to play a role in GPIbα-mediated signaling in an IgG-independent manner [70].

The interaction of fibrinogen- or fibronectin-bound *S. aureus* or *S. pyogenes* with platelet GPIIb/IIIa induces platelet aggregation in an antibody-dependent manner. Thus, *S. aureus* Clf A-mediated aggregation requires fibrinogen and antibody to bind to Clf A which in turn bind to GPIIb/IIIa and FcγRIIa respectively [65]. Similarly, vWf-bound bacteria such as *H. pylori* induce platelet aggregation in an FcγRIIa-dependent manner [33]. In this case the vWf binds to GPIbα and the antibody binds to FcγRIIa. Complement-dependent platelet aggregation is also antibody- and FcγRIIa-dependent [61].

In the case of *S. sanguinis* [68], *S. gordonii* and *S. pneumoniae* [56] aggregation is also FcγRIIa-dependent but there is no requirement for IgG to induce aggregation. This is analogous to the role of FcγRIIa in promoting cell signaling through GPIIb/IIIa [69] and GPIbα [70, 71, 72].

Bacterial toxins

As well as interacting with platelets through surface proteins, bacteria can also secrete toxins that can activate platelets [73]. *Porphyromonas gingivalis* is an oral pathogen that secretes a family of cysteine proteases known as gingipains [74]. These toxins can induce platelet aggregation by cleaving PAR-1 in a manner analogous to thrombin [75, 76]. *S. aureus* secretes a 34kDa pore toxin called α-toxin [77]. It is produced by almost all strains of *S. aureus*. It binds to the lipid bilayer of platelets creating a transmembrane pore and an influx of calcium [78, 79], which in turn triggers platelet activation in a manner analogous to the calcium ionophore A23187 [80]. Other pore-forming toxins include streptolysin O [81] from *S. pyogenes* and pneumolysin [82] from *S. pneumoniae* which activate platelets in a similar manner to α-toxin.

S. aureus and *S. pyogenes* can produce a super-family of toxins known as superantigens and Staphylococcal Superantigen-Like (SSL) toxins [83]. One of these (SSL5) has been shown to directly interact with GPIbα via the sLacNac residues that terminate its glycan chains [84]. SSL5 has also been reported in an abstract to bind directly to GPVI [85]. The binding of SSL5 to platelets triggered platelet activation and aggregation.

Effect of bacteria on platelet function

While it is clear that many bacteria can adhere to platelets and induce platelet aggregation it is important to confirm that this is not simply an *in vitro* artifact. Key elements here are evidence for signal generation in platelets in response to their interactions with bacteria; evidence of a response in models that better reflect *in vivo* conditions or evidence of response in animal models of disease.

Role of shear stress

Platelet aggregation and static adhesion studies are artificial systems that do not truly reflect the dynamic nature of the circulatory system. Platelets are routinely exposed to a range of shear stresses reflecting both venous and arterial conditions. Platelet function is sensitive to shear stress, for example, the interaction between GPIbα and vWf only occurs under conditions of high shear stress.

S. sanguinis and *S. gordonii* both interact with GPIbα and therefore it is not surprising that this interaction is shear-dependent. However, in contrast to the high-shear-dependent rolling of platelets over immobilized vWf, platelets roll over both streptococci under low shear conditions [26, 30]. Deletion of the serine-rich, highly glycosylated proteins, SrpA (*S. sanguinis*) or GspB/Hsa (*S. gordonii*) completely abolished rolling. Under low shear conditions, thrombus formation on *S. pyogenes* is antibody-, FcγRIIa-, fibrinogen- and GPIIb/IIIa-dependent as is platelet aggregation [86]. However, platelet aggregation induced by *S. aureus* is more complex with potential roles for Clf A and B and Fnbp A and B. Studies under shear conditions showed that thrombus formation only happened under high shear conditions (>800 s^{-1}) and that it was entirely dependent on Clf A as none of the other pro-aggregatory proteins could support thrombus formation. As with aggregation, thrombus formation was antibody-FcγRIIa and fibrinogen-GPIIb/IIIa-dependent [67].

Platelet signaling in response to pathogens

The ability of bacteria to generate intracellular signals upon binding to platelets is important in establishing a biological relevance for the interaction. There is a paucity of data on this due in part to the complex, multicomponent nature of the interactions.

Upon activation by *S. sanguinis*, platelets release their dense granules, which contain vasoactive substances including the adenosine nucleotides, ATP and ADP [87]. *S. sanguinis* also express an ectoATPase which hydrolases the released ATP to ADP [88, 89]. ADP binds to the platelet ADP receptors,

$P2Y_{12}$ and $P2Y_1$, to serve as an amplification step essential for stable aggregate formation. Further studies have characterized the signal induced by *S. sanguinis* and demonstrated that it is also cyclooxygenase- and thromboxane A_2-dependent [68]. More recently Pampolina and colleagues demonstrated that in the presence of IgG, *S. sanguinis* caused tyrosine phosphorylation of platelet FcγRIIa within 30 seconds followed by phosphorylation of PLCγ2, Syk and LAT. Subsequently there was tyrosine phosphorylation of PECAM-1 and the tyrosine phosphatase SHP-1 leading to dephosphorylation of PLCγ2, Syk and LAT. As aggregation progressed in to the early phase, platelets released thromboxane and contents of their dense granules acting to amplify and stabilize the platelet aggregate [90].

Keane and colleagues also demonstrated that platelet adhesion to immobilised *S. gordonii* resulted in tyrosine phosphorylation of the ITAM-bearing receptor, FcγRIIa, as well as phosphorylation of downstream effectors, Syk and PLCγ2. This signal resulted in platelet dense granule secretion, filopodia and lamellipodia extension, and platelet spreading. Inhibition of either GPIIb/IIIa or FcγRIIa completely abolished dense granule release and platelet spreading [91].

Streptococcus mitis has also been shown to bind to platelets in a GPIIb/IIIa- and GPIbα– independent manner, however no platelet activating signal was generated [92, 93]. Relatively little is known about the signal generated in platelets upon binding *S. aureus* other than it is cyclooxygenase- and thromboxane-dependent [94].

Phagocytosis

The presence of FcγRIIa on the platelets surface suggests that platelets may have the capacity to phagocytose as this receptor is important in immune complex clearance. Platelets have been shown to phagocytose immune complexes in an FcγRIIa-dependent manner and can also be phagocytosed themselves [95, 96] also in an FcγRIIa-dependent manner [47]. Platelets have been shown to enhance the phagocytosis of periodontal pathogens by

neutrophils [97]. Platelets can also directly phagocytose bacteria such as *P. gingivalis* [98, 99] and *S. aureus* [100, 101, 102]. However, often phagocytosis does not result in bacterial killing which has been suggested to be due to the structure of their vacuoles [100]. Bacteria can also get trapped in the space between platelets in an aggregate [99]. As a result, phagocytosis of bacteria by platelets can lead to the formation of a pool of viable bacteria present either intracellularly or within a thrombus which are protected from the immune system and plays a role in the pathogenesis of diseases such as infective endocarditis.

***Bacteria-platelet interactions* in vivo**

Several reports have investigated the interaction of bacteria with platelets under *in vivo* conditions. Mice infected with *S. aureus* develop platelet-rich thrombi in a process that is dependent on Clf A since administration of the fibrinogen-binding domain of Clf A prevented thrombus formation [103]. Dogs infected with *S. aureus* develop sepsis with an associated drop in platelet count [104]. Resistance to platelet anti-microbial peptides was a virulence factor in *S. aureus* for infective endocarditis [105] while hyper-production of α-toxin reduced the extent of *S. aureus* mediated endocarditis [106] presumably due to increased levels of anti-microbial peptides. *S. aureus* SraP is a virulence factor in infective endocarditis [31] as is wall teichoic acid [107]. *Lactococcus lactis* expressing either Clf A or Fnbp A were shown to be one-hundred times more infective than the wild-type *L. lactis* strain in an animal model of infective endocarditis [108]. However, MSCRAMMs have been shown to have only a modest role to play in *S. aureus*-mediated endocarditis in animal models [109, 110, 111] which is likely to be due to the presence of multiple platelet interacting proteins on the bacterial surface and the difficulty of generating a strain of *S. aureus* devoid of any interaction with platelets especially since complement formation can occur in the absence of these proteins. Thus, blockade of the complement receptor gC1qR was shown to be beneficial in *S. aureus* mediated endocarditis [112]. Deletion of the lysin gene from *S. mitis* significantly reduced endocarditis in a rat model [22]. There was evidence of increased rates of embolization in *H. pylori*-infected mice after laser-induced arterial damage [113].

Evidence from studies in mice suggests that in the case of *S. pneumoniae,* thrombocytopenia and DIC is due to a bacterial neuraminidase that removes sialic acid from platelet proteins making them substrates for the Ashwell receptor in the liver. Binding to the Ashwell receptor leads to clearance of platelets from the circulation resulting in thrombocytopenia [114].

H. pylori infection is associated with platelet activation in patients [115, 116]. Clinical studies have shown that *H. pylori* eradication therapy in patients with idiopathic thrombocytopenic purpura who are *H. pylori*-positive was effective at improving the platelet count [117, 118, 119, 120]. This suggests that on-going infection with *H. pylori* leads to platelet activation and subsequent thrombocytopenia.

There are several differences between human and rodent platelets most notably the absence of FcγRIIa. As FcγRIIa has been shown to play a significant role in the interaction of bacteria with human platelets the relevance of data from traditional mouse models of sepsis are questionable. As transgenic mice expressing FcγRIIa are now available it will be possible to use these to better understand the interaction of bacteria with platelets *in vivo*.

There is little data on the role of platelets in infection in humans but a study of patients with *S. pyogenes* toxic shock syndrome showed evidence of micro-thrombi in biopsies. These platelet aggregates formed in a process dependent on M1 protein, IgG and FcγRIIa [21] similar to that seen *in vitro*.

Conclusions

There is no doubt that platelets play an important role in the innate immune system. As the first responders to injury they are ideally placed to initiate an immune response to potential pathogens through secretion of anti-microbial peptides to kill bacteria and chemokines to attract other immune cells. Both the haemostatic and immune functions of platelets require platelet activation to occur. There are many different mechanisms by which bacteria can interact

with platelets including direct interactions with platelet receptors as well as the secretion of bioactive agents such as LPS. However, there is a paucity of *in vivo* data to identify the key mechanisms in infectious diseases such as sepsis. Is there a primary interaction driving the response or is it a combination of all of the interactions?

Some bacteria have developed resistance to the anti-microbial effects of platelets and have the ability to co-opt platelets into the infection process. By inducing platelet activation while resistant to the anti-microbial peptides they can become engulfed in a septic thrombus as occurs in infective endocarditis. They are then protected from the other cells of the immune system which allows them to persist in the circulation. Even when susceptible to anti-microbial peptides, rapid bacterial growth during sepsis leads to extensive platelet activation which in turn leads to DIC and shock. Thus, in these cases inhibition of platelet activation by bacteria may prevent some of the serious consequences of sepsis and infective endocarditis.

As each species of bacteria, and even individual strains, have different mechanisms for interacting with platelets it will in all likelihood prove impossible to target the bacteria as a mechanism to prevent platelet activation. However, there appears to be a limited number of platelet receptors involved making the platelet a better target. GPIIb/IIIa is an obvious target as it is important in *S. aureus*-induced platelet activation and there are approved inhibitors available. However, bleeding is a serious problem with these drugs and in a patient already thrombocytopenic due to sepsis they would further compromise the remaining platelets. GPIbα is another important target as it is important in streptococcal sepsis however, despite much effort there have been no approved GPIbα inhibitor. Aspirin could also be used to prevent platelet activation but as it also compromises platelet function and as some species of bacteria can induce platelet activation in a cyclooxygenase-independent manner it is of limited use. The most promising target is FcγRIIa as it plays a critical role in platelet activation induced by most species of bacteria as they either require bound IgG to induce aggregation or else

FcγRIIa is important in the activation process even if antibody is not required. Another advantage of targeting FcγRIIa is that it has minimal effects on normal platelet function, thus preserving platelet function and not causing bleeding. While there are no inhibitors of FcγRIIa at present the possibility of synthesizing such compounds has recently been demonstrated [121].

The conventional view of platelets has been that of anucleate cellular fragments that play a key role in haemostasis. The discovery of evidence for protein synthesis by platelets [122] suggested that they are more sophisticated than originally thought. We now have strong evidence that platelets are also key components of the innate immune system where they play important roles in infection and inflammation. While our understanding of the role of platelets in the immune system is far from complete we do see the possibility and potential benefits of specifically targeting the immune function of platelets in both autoimmune and infectious diseases.

Acknowledgements

Work in the authors' laboratories is supported by grants from Science Foundation Ireland, Health Research Board, Ireland, the British Heart Foundation and Wellcome Trust.

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Figure 1. Lag time to platelet aggregation. ADP induced platelet aggregation with a lag time of 15 sec, whereas *Streptococcus sanguinis* induces platelet aggregation with a lag time of 4 mins. Lag time is defined as the time taken from addition of agonist or bacteria to the first signs of platelet aggregation.

Figure 2. Summary of indirect interactions between bacteria and platelets. Different species of bacteria bind different plasma proteins which act as a bridge to their respective platelet receptor, thus triggering activation. ClfA; clumping factor A, FnbpA; fibronectin binding protein A, PA; protein A, IgG; immunoglobulin G, vWf; vonWillebrand Factor, C1q; complement 1q, GP; glycoprotein. Note that Protein A does not require antibody while *H. pylori* does.

Figure 3. Summary of direct interactions between bacteria and platelets. Different species of bacteria contain ligand mimetic motifs that act as agonist on platelet receptors. PadA; platelet adhesion protein A, IsdB; iron-regulated surface determinant B, SdrG; Serine aspartate repeat G, Hsa; haemaglutinin salivary antigen, GspB; glycosylated streptococcal protein B, SrpA; serine-rich protein A, LPS; lipopolysaccharide, GP; glycoprotein, TLR; Toll-like receptor

Table 1. Platelet bacterial interactions

Platelet receptor	Bacteria	Bacterial protein	Bridging protein
GPIIbIIIa	*S. epidermidis*	SdrG	Fibrinogen
	S. aureus	FnbpA/B	Fibronectin
	S. aureus	FnbpA/B	Fibrinogen
	S. aureus	ClfA	Fibronectin
	S. aureus	ClfA	Fibrinogen
	S. aureus	IsdB	Direct
	S. pyogenes	M1	Fibrinogen
	S. gordonii	PadA	Direct
	S. lugdunensis	Fbl	Fibrinogen
GPIbα	*S. sanguis*	SrpA	Direct
	S. gordonii	GspB/Hsa	Direct
	S. aureus	Protein A	vWf
	H. pylori	?	vWf
FcγRIIa	*S. aureus*	FnbpA/B	IgG
	S. aureus	ClfA	IgG
TLR2	*S. pneumoniae*	?	Direct
	?	Lipoprotein	Direct
TLR4	*E. coli*	LPS	Direct
gC1q-R	*S. sanguinis*	?	C1

Table 2. Platelet toxin interactions

Platelet receptor	Bacteria	Bacterial toxin
PAR1	*P. gingivalis*	**Gingipains**
GPIbα	*S. aureus*	**SSL-5**
GPVI	*S. aureus*	**SSL-5**
Phospholipids	*S. pneumoniae*	**Pneumolysin**
	S. aureus	**α-toxin**
	S. aureus	**Leukocidin**
	S. pyogens	**Streptolysin-O**