

# Start a fire, kill the bug: The role of platelets in inflammation and infection

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## Abstract

Platelets are the main players in thrombosis and hemostasis; however they also play important roles during inflammation and infection. Through their surface receptors, platelets can directly interact with pathogens and immune cells. Platelets form complexes with neutrophils to modulate their capacities to produce reactive oxygen species or form neutrophil extracellular traps. Furthermore, they release microbicidal factors and cytokines that kill pathogens and influence the immune response, respectively. Platelets also maintain the vascular integrity during inflammation by a mechanism that is different from classical platelet activation. In this review we summarize the current knowledge about how platelets interact with the innate immune system during inflammation and infection and highlight recent advances in the field.

## Keywords

Platelet, inflammation, infection, Kupffer cell, neutrophils, macrophages

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## Introduction

Platelets are small anucleated cell fragments patrolling the vasculature, and immediately respond to vessel breaches and restore hemostasis. In recent decades it has become clear that they play roles beyond hemostasis and also contribute to (thrombo-)inflammatory processes like those unfolding after stroke.<sup>1</sup> Furthermore, they also play an important role during infection by either directly interacting with pathogens or by recruiting and stimulating immune cells.<sup>2</sup> More recently, we have come to understand that platelets also maintain vascular integrity in inflamed vessels in a process different from classical hemostasis.<sup>3,4</sup> Acknowledging their multifaceted capabilities, platelets have lately been described as autonomous drones for hemostatic and immune surveillance.<sup>5</sup>

The finding that platelets form aggregates around bacteria is not new. In fact, one of the first descriptions of this process dates back to 1901<sup>6</sup> when Levaditi showed that platelets aggregated upon incubation with *Vibrio cholerae*; however, more systematic investigations on how bacteria cause platelet aggregation were only performed in the 1970s.<sup>7,8</sup> Platelet surface receptors enable direct platelet–bacteria or platelet–immune cell interactions.<sup>9</sup> Factors stored in platelet granules

that are released upon activation include cytokines, inflammatory mediators and antimicrobial peptides.<sup>10,11</sup> In this review we discuss how platelets, being among the first cells to respond to vessel injury, are at the front line of antimicrobial host defense which allows them to orchestrate the innate immune response (Figure 1).

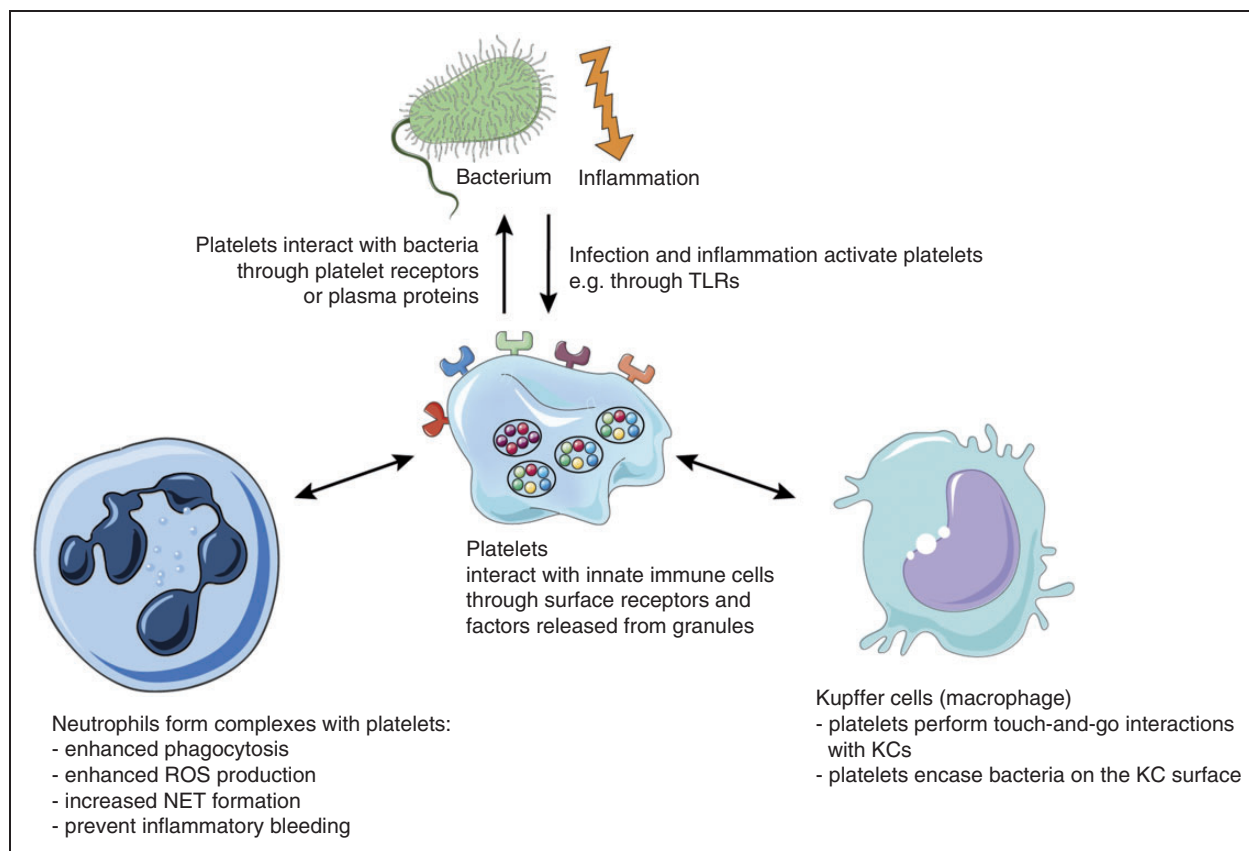
A lot of the findings presented here were discovered using experimental mouse models or knockout mouse lines. It is therefore worth to mention that there are some distinct characteristics between murine and human platelets such as their size, number and some histological features. However, they also share a lot of similarities, and mice offer an excellent model to study various aspects of platelet biology *in vivo*.<sup>12</sup>

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**Figure 1.** Platelets interact with bacteria and cells of the innate immune system. Platelets interact with bacteria directly through their surface receptors or indirectly through plasma proteins. Platelets orchestrate the immune reaction to inflammation and infection by direct interactions with cells of the innate immune system (neutrophils and Kupffer cells) or through the secretion of mediators.

## Platelets and sepsis

Severe sepsis—defined as infection in combination with acute organ dysfunction—is the leading cause of in-hospital death in the United States (US).<sup>13,14</sup> Estimations suggest there are 750,000 cases of severe sepsis every year in the US, with most of them requiring intensive care. Case numbers for patients with severe sepsis admitted to a hospital have seen a significant rise in recent years,<sup>14</sup> with the highest incidence rates reported for newborns younger than 12 months (5.3 per 1000) and senior patients older than 85 years (26.2 per 1000).<sup>15</sup> In the 1960s, some studies reported mortality rates of up to 80% for septic shock patients. Thanks to improvement in monitoring and therapy, this number has decreased significantly; however, it still remains at around 25% today.<sup>13,16</sup> In addition, total hospital costs for patients with severe sepsis have seen a steady increase, and it is now a major burden for the public health system with estimated annual costs of more than 24 billion USD in the US.<sup>14</sup>

During severe sepsis both pro- and anti-inflammatory responses occur simultaneously. The two processes aim at eliminating the pathogen and at the same time try to restrict the immune reaction to prevent excessive

damage. Both responses have to be delicately balanced to provide a response that is powerful enough to clear the pathogen and prevent secondary infections but also to minimize collateral tissue damage.<sup>13</sup>

Severe sepsis is often accompanied by disseminated intravascular coagulation and thrombocytopenia; however, the underlying mechanisms are incompletely understood.<sup>13,17</sup> It is well established, however, that there is extensive cross-talk between the inflammatory and coagulation pathways. Tissue factor (TF) is up-regulated by leukocytes, platelets, endothelial and smooth muscle cells, which triggers thrombin generation leading to a sustained pro-inflammatory and thrombotic response while at the same time dampening anticoagulant mechanisms and fibrinolysis.<sup>18,19</sup>

A recent study showed that thrombocytopenia led to severely impaired survival and enhanced bacterial growth in blood and lungs in a mouse model of pneumonia-derived sepsis.<sup>20</sup> Thrombocytopenia also caused hemorrhage at the site of infection, in line with previous results demonstrating that inflammation induces hemorrhage during thrombocytopenia.<sup>20,21</sup> A clinical study including more than 900 sepsis patients grouped them according to their platelet counts upon admission to the intensive care unit. Remarkably, patients with very low

or intermediate-low platelet counts showed significant increases in both 30-day mortality as well as cytokine levels and enhanced endothelial cell activation.<sup>17</sup> These results show that platelet count might be used as a prognostic marker during sepsis.

### Platelet receptors enable interactions with bacteria

Several platelet surface receptors are also involved in inflammation and infection in addition to thrombosis and hemostasis. Platelet-bacteria interactions can be either direct (bacterial surface proteins binding to a platelet receptor) or indirect (bacteria binding to a plasma protein, for example von Willebrand factor (vWF) or fibrinogen, which then binds to the respective platelet receptor).

#### Glycoprotein Ib (GPIb)

GPIb is exclusively expressed on the surface of platelets and precursor megakaryocytes. The interaction with its main ligand vWF is especially important for platelet adhesion under high shear conditions, for example in stenosed vessels or capillaries.<sup>22,23</sup> GPIb can also bind to serine-rich repeat proteins like the *Streptococcus sanguis* platelet adhesin called serine-rich protein A (SrpA) which enables binding to GPIb in a sialic acid-dependent manner.<sup>24</sup> *Staphylococcus aureus* protein A (Spa) facilitates indirect interaction with platelets through both soluble and immobilized vWF which then binds to platelets via GPIb.<sup>25,26</sup>

#### Integrin $\alpha$ IIb $\beta$ 3 (GPIIb/IIIa)

The platelet-specific integrin  $\alpha$ IIb $\beta$ 3 is the most abundant glycoprotein on the platelet surface and binds ligands that contain an arginine-glycine-aspartic acid (RGD) sequence such as fibrinogen, vWF, fibronectin and vitronectin.  $\alpha$ IIb $\beta$ 3 enables stable platelet-platelet interactions and adhesion to the extracellular matrix (ECM). Firm binding only occurs after a conformational change that puts  $\alpha$ IIb $\beta$ 3 in an activated or high-affinity state in which the RGD binding site is uncovered.<sup>27</sup> Binding of *Borrelia burgdorferi* to human platelets was shown to be mediated by  $\alpha$ IIb $\beta$ 3 and could be blocked by a synthetic RGD peptide.<sup>28</sup> Another bacterial protein that contains a RGD-like motif to interact with platelet  $\alpha$ IIb $\beta$ 3 is SdrG (Fbe) from *Staphylococcus epidermis*, which is present in many clinical strains and causes platelet aggregation which can be blocked using the  $\alpha$ IIb $\beta$ 3 antagonists abciximab or tirofiban, but also aspirin. SdrG also mediates indirect interactions through fibrinogen which in turn binds  $\alpha$ IIb $\beta$ 3 and the IgG receptor Fc $\gamma$ RIIIa.<sup>29,30</sup> Another well-characterized indirect interaction between platelets and bacteria is through

fibrinogen and clumping factors (Clf) on *S. aureus*. Like the Sdr proteins, ClfA and B both contain serine and aspartic acid dipeptide repeats (SD repeats) and bind fibrinogen to induce platelet aggregation via  $\alpha$ IIb $\beta$ 3.<sup>31,32</sup>

#### TLR2 / TLR4

TLRs recognize PAMPs, for example the bacterial cell wall components lipoteichoic acid (LTA) and LPS.<sup>33</sup> Platelets express TLR2 and TLR4 on their surface.<sup>34,35</sup> *Streptococcus pneumoniae* triggers platelet aggregation through TLR2, and  $\alpha$ IIb $\beta$ 3, independent of pneumolysin toxin, causes activation of the phosphoinositide 3-kinase (PI3-K) pathway and provokes dense-granule release.<sup>36</sup> In mice, LPS injection was shown to induce an increase in platelet binding to fibrinogen under flow. Furthermore, LPS administration caused thrombocytopenia through P-Selectin-independent neutrophil-mediated pulmonary platelet sequestration in wild type but not in TLR4-deficient mice.<sup>37</sup> Another study demonstrated that human platelets responded to LPS stimulation with release of soluble CD62p, epidermal growth factor, TGF $\beta$ , IL-8, platelet activating factor 4 (PAF4) and platelet-derived growth factor (PDGF)  $\alpha$  and  $\beta$ .<sup>37</sup> During hemolytic-uremic syndrome (HUS) caused by infection with enterohemorrhagic *Escherichia coli* (EHEC), platelets were activated by EHEC-LPS binding to TLR4.<sup>38</sup> Platelet TLR4 detects TLR4 ligands (LPS, but also others like high mobility group B1 (HMGB1) and heat-shock proteins) in the blood and causes platelets to bind to adherent neutrophils and the formation of neutrophil extracellular traps (NETs) in liver sinusoids and pulmonary capillaries, which facilitate bacterial capture during sepsis<sup>39</sup> but can also have detrimental effects such as causing vascular occlusion.<sup>40,41</sup>

#### (hem)ITAM receptors GPVI and CLEC-2

Platelets are activated by collagen through their main collagen receptor GPVI, which signals through an immunoreceptor tyrosine-based activation motif (ITAM) in the Fc receptor (FcR)  $\gamma$ -chain it forms a complex with.<sup>42,43</sup> The platelet C-type lectin receptor 2 (CLEC-2) binds podoplanin, which is highly expressed in type 1 lung alveolar cells, lymphatic endothelial cells and kidney podocytes, but absent from endothelial cells and platelets. CLEC-2 contains a single cytosolic YXXL motif known as a hemITAM that becomes phosphorylated upon receptor multimerization to enable signaling.<sup>44,45</sup> GPVI and CLEC-2 play important roles in thrombosis and hemostasis;<sup>42,46-48</sup> however, they are also involved in maintaining vascular integrity in inflamed vessels thus preventing inflammatory bleeding,<sup>3,49,50</sup> formation of cerebral blood vessels<sup>51</sup> and mediating blood/lymphatic vessel

separation.<sup>52</sup> A recent report showed that CLEC-2 mediates inflammation-triggered thrombosis after salmonella infection in the liver<sup>53</sup> through platelets interacting with podoplanin-expressing monocytes and Kupffer cells. Lately, a thrombosis-independent role for CLEC-2 during sepsis was described: Platelet CLEC-2 interacts with podoplanin expressed on inflammatory macrophages to regulate immune cell recruitment as well as the cytokine/chemokine storm following infection to limit organ damage.<sup>54</sup> In another study, *S. aureus*  $\alpha$ -toxin bound ADAM10 on the platelet surface to trigger platelet activation and platelet-neutrophil complex formation that enhanced neutrophil activity during sepsis.<sup>55</sup> ADAM10 is a metalloproteinase that cleaves the ectodomain of GPVI,<sup>56</sup> and in fact soluble GPVI (sGPVI) was released from platelets following incubation with *S. aureus*  $\alpha$ -toxin. Whether this demonstrates a direct interaction of ADAM10 with *S. aureus* or a potential role for sGPVI needs further investigation. Moreover, GPVI-deficient mice showed increased bacterial growth in lungs and distant body sites after pneumonia-derived sepsis as well as reduced platelet activation and platelet-leukocyte complex formation in the bronchoalveolar space.<sup>57</sup> Interestingly, in a recent clinical study, sGPVI was identified as a marker for platelet activation and predictive for the occurrence of sepsis and overall survival in patients with thermal injury.<sup>58</sup> GPVI is also critically involved in the formation of platelet microparticles—submicrometer vesicles shed from activated platelets—that can have pro-inflammatory effects, for example in patients with rheumatoid arthritis.<sup>59</sup> Recently it was shown that microparticles shed from activated platelets lose GPVI expression while maintaining CLEC-2,<sup>60</sup> contributing to sGPVI levels. Indeed, sGPVI levels in plasma from patients with rheumatoid arthritis were significantly increased compared with healthy controls.

### Platelet granule-derived factors and their impact on innate immunity

Upon activation, platelets release a plethora of different factors stored in two major types of granules:  $\alpha$ -granules and dense granules.  $\alpha$ -Granules are highly abundant, with 50–80 granules per mouse platelet,<sup>61,62</sup> while dense granules are considerably less abundant with 5–6 granules per platelet.  $\alpha$ -Granules contain more than 300 different membrane and soluble proteins, which are recruited to the plasma membrane or secreted upon platelet activation, respectively, and are involved in processes such as platelet adhesion, coagulation, thrombo-inflammation, wound healing, tumor growth, angiogenesis and antimicrobial host defense.<sup>61,63,64</sup>

There is increasing evidence that platelets contribute to the onset and spread of inflammation.<sup>65</sup> Platelets

adhere to the activated endothelium or form complexes with immune cells to activate, attract or differentiate other immune cells by several mechanisms. Many platelet-derived factors contribute to shaping the inflammatory response, and one of the most important ones is P-Selectin, which is exposed on the platelet surface upon activation and mediates interactions of platelets with immune cells and the endothelium. Platelet P-Selectin binds to P-Selectin glycoprotein ligand-1 (PSGL1) on endothelial or immune cells, thereby enabling platelets to bind to the inflamed endothelium, to recruit circulating monocytes, neutrophils and lymphocytes and to initiate an inflammatory response at the site of injury. Importantly, blocking P-Selectin or PSGL-1 using antibodies almost completely abolished platelet tethering, rolling and adhesion on activated endothelium.<sup>66</sup> In a mouse model of acute lung injury, blocking platelet P-Selectin reduced the number of platelet-neutrophil complexes, improved gas exchange, reduced neutrophil recruitment and permeability and prolonged survival of the animals.<sup>67</sup>

Upon activation, platelets secrete numerous chemokines, including CXCL1, CXCL4, CXCL5, CXCL7, CXCL8, CXCL12, CCL2, CCL3 and CCL5.<sup>10</sup> The most abundant platelet chemokine CXCL7 is present in several variants: platelet basic protein, connective tissue-activating peptide III (CTAP-III),  $\beta$ -thromboglobulin ( $\beta$ -TG) and neutrophil-activating peptide-2 (NAP-2), and all of them are generated by proteolytic cleavage from a precursor protein.<sup>10,61</sup> However, the only variant that possesses chemotactic activity is NAP-2.<sup>68</sup> It was shown that both CTAP-III and NAP-2 induce neutrophil adhesion to human umbilical vein endothelial cells (HUVECs); however, only NAP-2 triggered neutrophil transendothelial migration.<sup>69</sup> Interestingly, CXCL7 is also involved in the recruitment of circulating endothelial progenitor cells after arterial injury through its receptor CXCR2, indicating that CXCL7 secreted by platelets may contribute to revascularization after vessel injury.<sup>70</sup>

Platelets are a major source of CCL5 (RANTES) in the circulation, as its concentration was highly correlated to platelet counts in a study of patients with hematological malignancy undergoing chemotherapy.<sup>71</sup> Platelet-derived CCL5 contributes to recruiting monocytes to the vessel wall. In a mouse model of atherosclerosis, activated platelets were shown to deliver CCL5 and CXCL4 (platelet factor 4, PF4) to the surface of both monocytes and endothelial cells in atherosclerotic lesions in a P-Selectin-dependent manner.<sup>72</sup> It was further shown that CXCL4 facilitates CCL5 oligomerization and amplifies its effect on monocyte recruitment.<sup>73</sup>

Platelets (and their precursors megakaryocytes) are the exclusive source of CXCL4 (PF4). That platelets have an abundance of CXCL4 is strikingly demonstrated by a 1000-fold increase in the serum



concentration after thrombin stimulation.<sup>61,74</sup> Although CXCL4 lacks chemotactic activity,<sup>75</sup> it causes firm neutrophil adhesion to endothelial cells and degranulation. While the first process is a direct effect of Src kinase activation, the latter requires costimulation, for example by TNF through p38 MAP kinase and PI3 kinase.<sup>76</sup> Furthermore, CXCL4 triggers several functions in monocytes, including phagocytosis, respiratory burst, survival and cytokine secretion. CXCL4-initiated respiratory burst was shown to depend on rapid activation of the PI3 kinase, Syk and p38 MAP kinase. By contrast, monocyte differentiation and survival is mediated by CXCL4-mediated delayed Erk activation approximately 6 h after stimulation.<sup>77,78</sup> CXCL4 released from platelets is also capable of inducing differentiation of monocytes into macrophages and prevents them from undergoing spontaneous apoptosis in culture.<sup>79</sup> In combination with IL-4, CXCL4 induces a rapid differentiation of monocytes into specialized APCs that stimulates lymphocyte proliferation and lytic NK activity while inducing only moderate cytokine release.<sup>80</sup>

CXCL4 also acts on other cell types such as endothelial cells. Platelet-derived CXCL4 inhibited fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor function by blocking the binding to their respective receptors. Furthermore, both CXCL4 and its variant CXCL4L1 potently inhibit chemotaxis and proliferation of endothelial cells as well as *in vitro* and *in vivo* angiogenesis.<sup>81,82</sup> CXCR3-B, a splice variant of CXCR3, was shown to be a receptor for CXCL4 and might be involved in the angiostatic activity of CXCL4 released by platelets<sup>83</sup> that causes inhibition of endothelial cell growth.

### Platelets as phagocytes and platelet-derived antimicrobial peptides

We know that platelets express TLR4<sup>36</sup> and other receptors which enable them to detect and bind to bacteria, which raises the question of their capability of phagocytosing bacteria. Indeed, electron microscopic studies demonstrated that activated platelets engulf *S. aureus* in vacuoles and appear to secrete granule content into the vacuole.<sup>84</sup> However, whether platelets actually killed the bacteria or transferred them to professional phagocytes was not clarified in this study. Interestingly, in an earlier report, Yeaman and colleagues isolated and characterized cationic proteins from rabbit platelets that displayed *in vitro* microbistatic or microbicidal activity against *S. aureus*, *Escherichia coli* and *Candida albicans*.<sup>85</sup> In another study, thrombin-induced platelet microbicidal protein (tPMP-1) potently lysed *S. aureus* during logarithmic-phase growth.<sup>86</sup> Releasate from thrombin-activated platelets reduced the number of adherent bacteria in a rabbit model of infective endocarditis using

*Streptococcus sanguis*.<sup>87</sup> Using the same model, experimentally induced thrombocytopenia led to higher densities of streptococci within vegetations as well as higher total number of bacteria per valve.<sup>88</sup> Another group of antibacterial proteins found in platelets is called thrombocidins (TCs). TC-1 and TC-2 were able to kill *Bacillus subtilis*, *E. coli*, *S. aureus* and *Lactococcus lactis*<sup>89</sup> using a mechanism that did not lyse the bacterial cell wall, indicating that they act differently than tPMP-1. Platelets were also shown to bind *Plasmodium falciparum*-infected erythrocytes and kill the parasite inside the cell.<sup>90</sup> Treating the platelets with aspirin or a P2Y<sub>1</sub> antagonist rendered them incapable of killing the parasite. Accordingly, treating mice with antiplatelet Abs or aspirin resulted in lower overall survival after infection with *Plasmodium chabaudi*. Lately, *E. coli* were found to be killed by human platelets through a process involving CXCL4 and FcγRIIA: Anti-CXCL4/polyanion Abs opsonized *E. coli* coated with platelet-derived CXCL4. The Ab complex was then detected by platelet FcγRIIA,<sup>91</sup> causing platelets to cover the opsonized bacteria and release antimicrobial factors in a concerted way to effectively kill them.

### Platelet interactions with innate immune cells in infection and inflammation

Platelets interact with a number of key players during infection and inflammation, namely macrophages (e.g. Kupffer cells in the liver), neutrophils, monocytes, NK cells, dendritic cells and components of the complement system. Here, we focus on reviewing their interactions with Kupffer cells, neutrophils, the complement system and how they maintain vascular integrity during inflammation and infection.

#### Kupffer cells

The liver is not only the largest internal organ with important roles in detoxification and metabolism, it is also the first line of defense against pathogens present in the blood stream. It filters about a third of the body's total blood volume each minute and contains the largest population of phagocytes in the body.<sup>92</sup> The liver-resident macrophage population known as Kupffer cells (KCs) are very large, immobile cells that reside in the sinusoidal space where they scan for foreign objects in the blood. More than 25 years ago, Endo and colleagues discovered that LPS injection into mice caused an increase in hepatic serotonin levels which was independent of mast cells and correlated with a drop in the number of circulating platelets. They found that LPS application caused accumulation of non-activated platelets in the sinusoidal space where they frequently interacted with KCs.<sup>93</sup> Subsequent studies showed that while aspirin or heparin did not affect

serotonin accumulation in the liver, KC depletion using clodronate liposomes almost completely abolished platelet and serotonin accumulation.<sup>94</sup> This suggested that LPS stimulation causes platelet retention in the liver through a process that is different from classical platelet aggregation and that involves KCs.

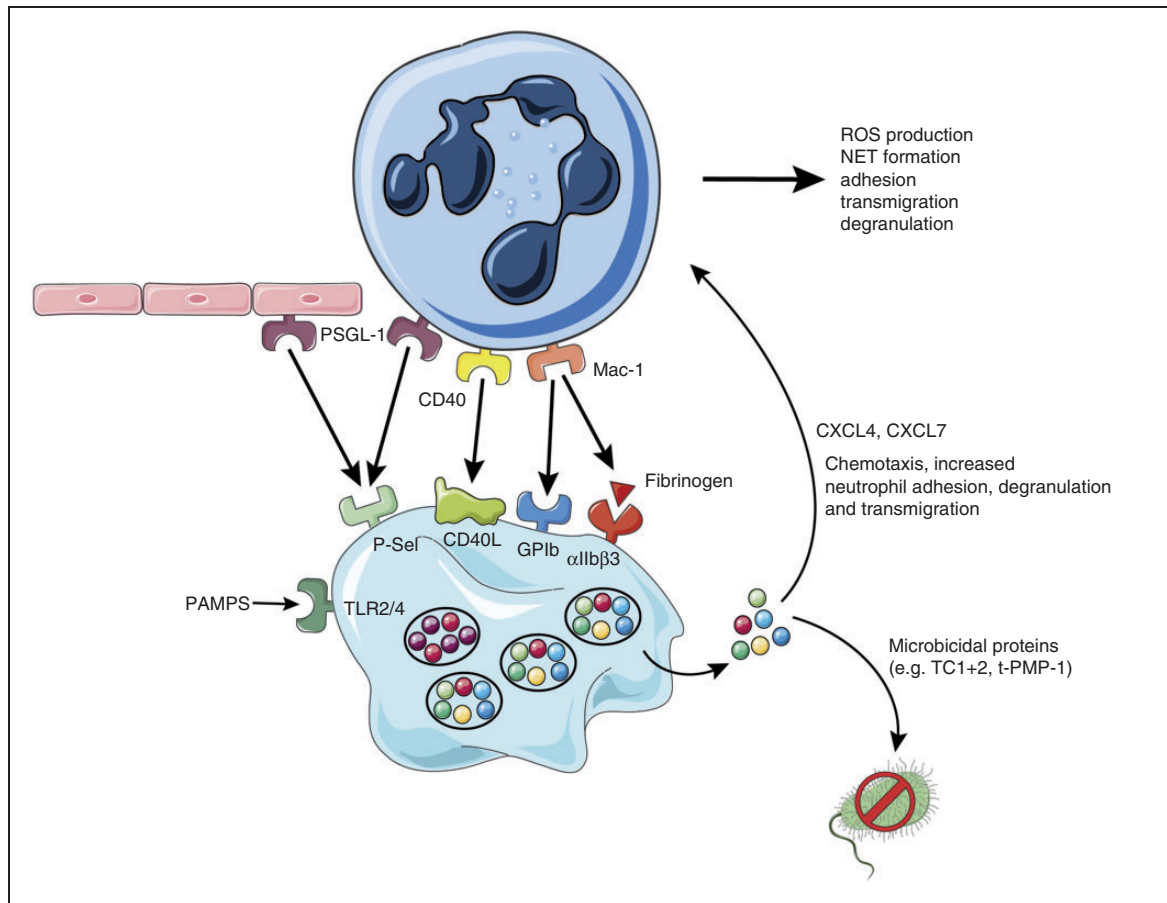
We now know that KCs rapidly capture a striking quantity of pathogens from the bloodstream in a process that often involves platelets. KCs were shown to bind *B. burgdorferi* and prevent them from using sinusoidal endothelial cells to gain access to the extravascular space.<sup>95</sup> It was also demonstrated that methicillin-resistant *S. aureus* (MRSA) is primarily sequestered and killed by KCs through an interaction of the complement receptor of the immunoglobulin superfamily (CRIg) with LTA on the bacterial surface.<sup>96</sup> A minority of staphylococci, however, can overcome the antimicrobial activity of the KCs to survive and proliferate inside this intracellular niche.<sup>97</sup> Importantly, KCs were found to collaborate with platelets to eradicate blood-borne infections with *Bacillus cereus* and MRSA.<sup>98</sup> In the naïve mouse liver, platelets performed touch-and-go interactions through GPIb with vWF constitutively expressed on KCs. Upon infection, KCs captured bacteria and platelets rapidly adhered and formed aggregates around them in an integrin  $\alpha$ IIB $\beta$ 3-dependent way to contain the bacterium. This suggested an important role for platelets in KC-mediated bacterial clearance. Indeed, platelet depletion or lack of GPIb resulted in severely increased mortality in mice following infection. Importantly, opsonization with complement factor C3 was necessary for successful bacterial clearance, indicating a complex interplay between KCs, platelets and the complement system during bacterial clearance in the liver. Another group investigating the clearance of *Listeria monocytogenes* bloodstream infections by KCs discovered a dual-track mechanism consisting of a slow clearance of bacteria-platelet complexes that requires platelet GPIb, CRIg and C3 opsonization as well as a fast clearance of free bacteria independent of complement and platelets that requires scavenger receptors.<sup>99,100</sup> The authors hypothesized that the slow clearance allows a small number of platelet-bacteria complexes to remain in the circulation long enough to be detected by splenic CD8 $\alpha$ <sup>+</sup> dendritic cells to launch an antibacterial cytotoxic T cell response.

## Neutrophils

It is well established that platelets interact with neutrophils during inflammation and infection. Indeed, circulating platelet-neutrophil complexes (PNC) have been found in a variety of diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, stroke and severe sepsis.<sup>101–102</sup> The effect of platelets binding to neutrophils includes increased

neutrophil adhesion to the endothelium, increased reactive oxygen (ROS) production and NET formation (Figure 2). P-Selectin, which upon activation is recruited to the platelet surface from  $\alpha$ -granular stores, seems to be the most important platelet surface receptor for platelet-neutrophil interactions. Platelet P-Selectin binds to the high-affinity counter-ligand PSGL-1 on neutrophils.<sup>104,105</sup> P-Selectin-deficient mice show severe leukocyte defects, for example abrogated leukocyte rolling as well as delayed neutrophil recruitment and reduced neutrophil extravasation.<sup>106,107</sup> Using P-Selectin-deficient mice or treating mice with an anti-P-Selectin Ab and subjecting them to different models of lung inflammation resulted in reduced neutrophil recruitment and less lung damage.<sup>108,109</sup> However, all those studies looked at global P-Selectin deficiency in which the individual contribution of endothelial and platelet P-Selectin are difficult to isolate. In later studies it was shown that platelet depletion significantly inhibits neutrophil recruitment to the site of inflammation in a zymosan-induced peritonitis and LPS-induced lung inflammation model.<sup>110</sup> In inflamed glomerular capillaries, platelets are essential for leukocyte adhesion via a non-classical cascade that involves platelet P-Selectin binding to endothelial PSGL-1 as well as  $\beta$ 2 integrin/ICAM-1 and nonrolling interactions.<sup>111</sup> Depletion of neutrophils and platelets reduced urinary protein excretion induced by anti-glomerular basement membrane Abs, underlining their importance for the development of renal injury. In addition, it was shown that platelet P-Selectin is important for neutrophil recruitment into the outer and inner medulla during acute post-ischemic renal failure.<sup>112</sup> In another paper, Sreeramkumar et al. showed that in inflamed vessels, neutrophils scan for the presence of activated platelets using PSGL-1 clusters. Migration and NET formation only occurred once activated platelets had bound.<sup>113</sup> In a mouse model of multiple sclerosis called experimental autoimmune encephalomyelitis (EAE), platelet depletion significantly improved the disease state and slowed its progression through reduced recruitment of leukocytes to the inflamed central nervous system and attenuated inflammation. More specifically, targeting GPIb or  $\alpha$ IIB $\beta$ 3 led to a pronounced improvement of EAE outcome.<sup>114</sup>

Interestingly, platelets not only express P-Selectin, but also its counter-receptor PSGL-1 which mediates platelet-endothelium interaction.<sup>115,116</sup> Furthermore, platelet GPIb was also shown to bind to (endothelial) P-Selectin,<sup>117</sup> thereby allowing platelets to scan for the activation status of the vasculature. In addition, platelets can stimulate the secretion of Weibel-Palade bodies from endothelial cells and leukocyte rolling through P-Selectin.<sup>118</sup> Therefore, it seems that platelets can interact with the endothelium to pave the way for leukocytes to bind to both platelets and the endothelium.



**Figure 2.** Platelets form complexes with neutrophils to potentiate their activity. Platelets interact with neutrophils through multiple receptors: Activated platelets express P-Selectin on their surface which binds PSGL-1 on neutrophils and endothelial cells, CD40L is expressed on the platelet surface upon activation and binds CD40, Neutrophil Mac-1 binds platelet GPIb as well as  $\alpha$ IIb $\beta$ 3. Together these interactions promote ROS production, NET formation, adhesion, transmigration and degranulation of neutrophils. Upon activation (e.g. through PAMPs binding to TLRs), platelets release microbicidal proteins like TC1+2 which can kill bacteria. They also secrete large quantities of CXCL4 and CXCL7 that promote neutrophil adhesion, degranulation and transmigration.

P-Selectin/PSGL-1 interactions between activated platelets and neutrophils in the inflamed vasculature promote further interactions through  $\beta$ 2 integrins (CD18), especially Mac-1 ( $\alpha$ M $\beta$ 2, CD11b/CD18) which follows a three-step process that involves binding to fibrinogen/GPIb and outside-in-signaling through Src family kinases.<sup>119,120</sup> In addition, activated platelets express intercellular adhesion molecular-2 (ICAM-2, CD102) which enables the formation of firm and shear resistant platelet-neutrophil-complexes under flow conditions by binding to  $\beta$ 2 integrins.<sup>121,122</sup>

Neutrophil accumulation on activated platelets under flow conditions also involves interactions of Mac-1 with fibrinogen bound to platelet  $\alpha$ IIb $\beta$ 3, as found by using blocking Abs against the major platelet integrin. Furthermore, platelets from patients with Glanzmann thrombasthenia harboring genetic defects in their *ITGA2B* or *ITGB3* genes that cause impaired  $\alpha$ IIb $\beta$ 3 function or expression<sup>123</sup> demonstrated

significantly reduced neutrophil adhesion to platelets under flow compared with healthy controls.<sup>124</sup>

Platelets are a major source of CD40 ligand (CD40L), which they express on their surface upon activation.<sup>125</sup> Patients with diabetes, ischemic stroke or acute coronary syndromes often show elevated levels of circulating soluble CD40L. Stimulation of endothelial cells through CD40 by platelet CD40L induces recruitment of neutrophils, likely via platelet P-Selectin and neutrophil Mac-1 expression. Furthermore, CD40L increased neutrophil oxidative burst and degranulation.<sup>126,127</sup>

Indeed, neutrophils in PNCs show increased activation, CD11b expression, phagocytosis and ROS production compared with free neutrophils.<sup>128</sup> Upon stimulation of platelets, the activation state of neutrophils in PNCs was even more pronounced.<sup>128,129</sup> Interestingly, incubation with resting platelets was able to restrict neutrophil activation, indicating that there is a strong interconnection between the activation

state of platelets and neutrophils in PNCs, which is most likely mediated through P-Selectin.<sup>129,130</sup>

Besides phagocytosis, neutrophils possess another clever way to capture bacteria through the formation of NETs. During NET formation, neutrophils expel large amounts of chromatin and granular proteins (e.g. elastase and myeloperoxidase (MPO)), thereby forming extracellular fibers to immobilize and kill bacteria.<sup>131,132</sup> Initially, it was thought that all neutrophils die during NETosis; however, early in infection, live neutrophils in fact release NETs to prevent bacterial dissemination.<sup>133</sup> While this may sound counter-intuitive at first, we know that red blood cells or platelets live without a nucleus for several days and that neutrophils whose nucleus has been removed retain their ability to crawl, transmigrate, phagocytose and kill bacteria at least for a short time.<sup>134</sup>

Platelets are critically involved in NET formation through their TLR4 receptor that can detect PAMPs in the bloodstream. Platelet activation through TLR4 causes them to bind to neutrophils adhering primarily to liver sinusoids and pulmonary capillaries, causing neutrophil activation and NET formation.<sup>39</sup> Significantly, adding plasma from patients with severe sepsis to platelets and neutrophils from healthy donors triggered TLR4-dependent PNC formation. Moreover, neutrophils migrate to liver sinusoids during sepsis to release NETs and prevent bacterial dissemination to other organs by a mechanism that requires PNC formation through LFA-1 (CD11a/CD18).<sup>135</sup> Platelets were shown to form aggregates around *S. aureus* thereby limiting their growth. In addition, human platelets also release an antimicrobial peptide called human  $\beta$ -defensin-1 (hBD-1) after stimulation with *S. aureus*  $\alpha$ -toxin. hBD-1 significantly impairs bacterial growth but was also shown to induce robust NET formation.<sup>136</sup>

Interestingly, NETs can also be found under sterile inflammation conditions in the lungs and plasma of patients with transfusion-related acute lung injury (TRALI). Targeting platelet activation using aspirin or an  $\alpha$ IIb $\beta$ 3 inhibitor decreased NET formation and lung injury in a mouse model of TRALI.<sup>137</sup> Blocking Mac-1 but not LFA-1 during acute lung injury also significantly reduced the amount of NET formation and lung injury.<sup>138</sup> Platelets are critically involved in the propagation of deep vein thrombosis (DVT) by promoting leukocyte accumulation and NET formation, which provides a prothrombotic surface through its decoration with TF.<sup>40</sup> Importantly, treating mice with DNase significantly reduced NET formation and DVT growth. A recent publication showed that both mice and humans harbor endogenous DNases that help to contain NET formation in the host.<sup>41</sup> Serum from mice deficient in DNase1 or DNase1L3 was able to degrade NETs; however, deficiency in both DNases completely abrogated NET-degrading capacity. Double-deficient animals subjected to chronic

neutrophilia or septicemia showed a high mortality due to vascular occlusion through “pure” NET clots independent of classical hemostasis or thrombus formation.

Platelets were recently found to be capable of actively probing their local environment and migrating using actomyosin-generated forces.<sup>139</sup> While migrating they act as cellular scavengers that collect bacteria both *in vitro* and *in vivo*. Platelet–bacteria bundles generated this way facilitate phagocytosis by neutrophils as well as NET formation.

## Complement

The complement system facilitates lysis of pathogens and damaged cells by forming a pore in the target cell membrane through the membrane attack complex. Platelets are capable of activating both the classical and alternative complement pathway; however, the mechanism is still incompletely understood.<sup>140</sup> It is known that activated human platelets express gC1qR—a receptor for C1q and the first factor of the classical complement pathway<sup>141,142</sup> which also binds to other ligands such as SpA expressed on the surface of *S. aureus*.<sup>143,144</sup> Platelet granules store complement C3 and C4 precursor but also C1 inhibitor, which indicates that platelets might in fact regulate the complement response.<sup>61</sup> Chondroitin sulfate released from activated platelets causes complement activation through interactions with C1q.<sup>145</sup> In addition, platelet P-Selectin can bind C3b and trigger the alternative complement pathway.<sup>140</sup>

*S. sanguis* induces platelet aggregation in a complement-dependent way with a lag time that can be explained by the time needed for the assembly of the C5b-9 complex on the bacterial surface.<sup>146</sup> *S. aureus* ClfA exhibits an alternative route to bind platelets that is fibrinogen-independent and involves Fc $\gamma$ RIIa and the assembly of complement proteins as well as a complement receptor.<sup>147</sup>

The complement factors C1q, C4, C3, and C9 bind TRAP (thrombin receptor-activating peptide)-activated platelets without, however, activating the complement cascade, indicating that under physiological conditions there is no activation of the complement system on the platelet surface.<sup>148</sup> Assembly of the lytic terminal complement complex C5b-9 on the platelet plasma membrane can activate platelets and induce platelet procoagulant activity.<sup>149</sup> Patients suffering from HUS display hemolytic anemia, acute kidney failure, complement system activation and microvascular thrombosis leading to thrombocytopenia.<sup>150</sup> During HUS, endothelial and complement system activation lead to vWF release from endothelial Weibel–Palade bodies as well as P-Selectin and TF recruitment in a C3a or C5a-dependent way that leads to platelet adhesion and establish the prothrombotic state.<sup>150–152</sup>

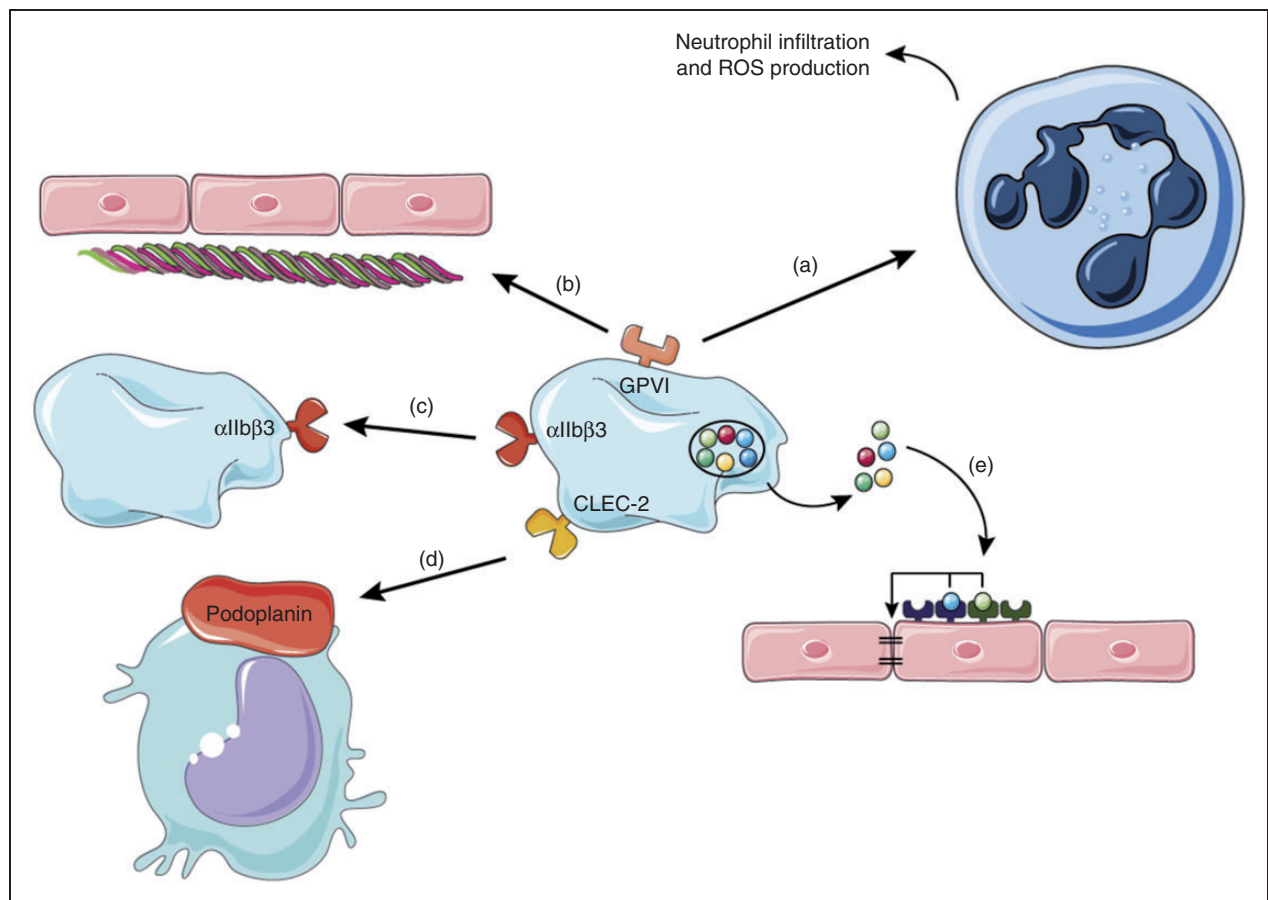


### Platelets maintain vascular integrity during inflammation

In patients with immune thrombocytopenia (ITP), the immune system wreaks havoc on endogenous platelets through antiplatelet Abs against major platelet receptors, in most cases GPIb and  $\alpha$ IIb $\beta$ 3.<sup>153</sup> This results in a significantly reduced number of circulating platelets ( $<100 \times 10^9/L$ ), causing varying degrees of bleeding culminating—in some cases—in intracranial hemorrhages.<sup>154</sup> Often the degree of thrombocytopenia observed in ITP patients does not predict the severity of bleeding,<sup>155</sup> indicating that thrombocytopenia alone is not sufficient to cause bleeding and that an additional trigger—such as inflammation—is needed.

In 2008, Goerge et al. showed that inflammation induces hemorrhage in thrombocytopenic mice.<sup>21</sup> Using multiple models to induce local inflammation in the skin, brain and lung, they observed no signs of bleeding in mice with normal platelet counts. In striking

contrast, when using thrombocytopenic mice, they observed massive hemorrhage at the site of inflammation. Remarkably, as little as 5% of the baseline platelet count was sufficient to significantly reduce bleeding. Interestingly, similar results were obtained in a model of pneumonia-derived sepsis using *Klebsiella pneumoniae*: Thrombocytopenic mice displayed increased bacterial growth and hemorrhage in the lung.<sup>20</sup> Later, the importance of platelet ITAM signaling downstream of GPVI and CLEC-2 in maintaining vascular integrity during skin and lung inflammation was demonstrated.<sup>49</sup> Recent findings show that the CLEC-2 ligand podoplanin is upregulated on macrophages and other extravascular cells during skin inflammation, indicating that in the absence of GPVI, binding of platelet CLEC-2 to podoplanin-expressing cells contributes to limiting bleeding in the inflamed skin (Figure 3).<sup>156,157</sup> The study also showed that vascular integrity during lung inflammation was partially dependent on GPIb.



**Figure 3.** Platelets maintain vascular integrity during inflammation. During local inflammation (e.g. in the skin), platelet GPVI plays a dual role: On the one hand it promotes neutrophil infiltration and ROS production which causes tissue damage (a) and on the other hand it binds to the extracellular matrix protein collagen which gets exposed and facilitates platelet adhesion to restore vascular integrity (b). In the ischemic brain  $\alpha$ IIb $\beta$ 3 facilitates platelet–platelet interactions to prevent intracranial hemorrhage (c). Platelets can also bind to podoplanin, expressed on inflammatory macrophages via CLEC-2 (d). Factors secreted from platelet granules support cerebral hemostasis after stroke, for example by acting on endothelial cell receptors that stabilize cellular junctions (e).

It was suggested that platelet granule secretion following platelet activation through the aforementioned pathways could be part of the mechanism that maintains vascular integrity.<sup>21,49,50,158</sup> However, recent findings demonstrate that platelet  $\alpha$ - and dense-granule contents are dispensable for maintaining vascular integrity during acute inflammation in the skin and lung.<sup>159</sup> Strikingly, when mice lacking platelet  $\alpha$ - and dense-granule secretion were subjected to transient middle cerebral artery occlusion (tMCAO), this resulted in significantly impaired hemostasis in the ischemic brain, causing increased intracranial hemorrhage and 50% mortality comparable to that observed in mice treated with  $\alpha$ IIB $\beta$ 3-blocking Abs.<sup>160</sup> This is especially interesting, since mice with a single deficiency in either platelet  $\alpha$ - or dense-granule secretion did not show signs of spontaneous hemorrhage during thrombosis,<sup>64,161</sup> thrombo-inflammation after stroke<sup>63,64</sup> or tumor metastasis.<sup>162,163</sup> This indicates that factors from both  $\alpha$ - and dense granules are necessary to maintain cerebral hemostasis after tMCAO; for example platelet-derived angiopoietin-1 or serotonin—which were previously shown to prevent intra-tumor hemorrhage<sup>165</sup>—might play a role in this setting. Of note, angiopoietin-1 was also shown to be critical for the maintenance of vascular integrity and survival in a mouse model of cerebral malaria.<sup>165</sup> In addition, brain endothelial cells express the P2Y2 receptor that binds nucleotides such as ADP and ATP, which could also contribute to permeability.<sup>166</sup> Indeed, platelet-derived ATP was shown to enable tumor cell trans-endothelial migration and metastasis via P2Y2.<sup>163</sup>

During inflammatory bleeding in the skin, neutrophil extravasation and RBC loss colocalize,<sup>167</sup> and inhibiting neutrophil capturing, adhesion and crawling on the endothelial cell layer significantly reduced hemorrhage. Furthermore, neutrophil diapedesis opens endothelial junctions via dephosphorylation of VE-cadherin during skin inflammation. Interestingly, platelet GPVI on the one hand enhances neutrophil infiltration and ROS production, thereby causing more endothelial damage,<sup>50</sup> while on the other hand it enables platelets to adhere to binding sites exposed by neutrophils. GPVI therefore has a Janus face in this process: It contributes to the pro-inflammatory role of platelets while at the same time helps to repair the damage inflicted by neutrophils and thereby maintains vascular integrity.<sup>168</sup>

## Conclusion

The roles of platelets besides those in thrombosis and hemostasis have long been neglected, but today we know that they also contribute to inflammation during sepsis, thrombo-inflammation, atherosclerosis and stroke. In most of these cases, platelets present themselves as a most versatile actor: They can form complexes with neutrophils and enhance their

phagocytosis, ROS production and NET formation capacity, encase bacteria on the surface of KCs to assist with their destruction or confront pathogens on their own by acting like a wannabe-phagocyte.

First reports of platelets aggregating around bacteria are more than 100 years old; however, only recently we have started to understand the complex interplay between platelets and the cells of the innate immune response during inflammation and infection. Platelets interact with bacteria by direct interactions between platelet receptors and proteins on the bacterial surface. Upon activation platelets release a plethora of factors, for example microbicidal agents but also factors that modulate the innate immune response.

NET formation by neutrophils is a powerful tool to capture and destroy bacteria and it has become clear that platelets critically contribute to this process. A very new concept is that platelets assist in bacterial clearance in the liver through KCs and the complement system, and we will probably see a lot of exciting new findings in this field in the near future.

Another emerging role for platelets is in maintaining vascular integrity during inflammation through an organ-specific process that is independent of classical activation and involves platelet receptors as well as the content of  $\alpha$ - and dense granules.

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## References

1. Nieswandt B, Kleinschnitz C and Stoll G. Ischaemic stroke: A thrombo-inflammatory disease? *J Physiol* 2011; 589: 4115–4123.
2. Deppermann C and Kubes P. Platelets and infection. *Semin Immunol* 2016; 28: 536–545.
3. Ho-Tin-Noé B, Boulaftali Y and Camerer E. Platelets and vascular integrity: How platelets prevent bleeding in inflammation. *Blood* 2018; 131: 277–288.

4. Deppermann C. Platelets and vascular integrity. *Platelets* 2018; 00: 1–7.
5. Li JL, Zarbock A and Hidalgo A. Platelets as autonomous drones for hemostatic and immune surveillance. *J Exp Med* 2017; 214: 2193–2204.
6. Levaditi C. Et des organismes vaccines contre le vibron cholérique. *Ann Inst Pasteur* 1901; 15: 894–924.
7. Clawson CC and White JG. Platelet interaction with bacteria. I. Reaction phases and effects of inhibitors. *Am J Pathol* 1971; 65: 367–380.
8. Clawson CC and White JG. Platelet interaction with bacteria. II. Fate of the bacteria. *Am J Pathol* 1971; 65: 381–397.
9. Kerrigan SW. The expanding field of platelet-bacterial interconnections. *Platelets* 2015; 26: 293–301.
10. Gleissner CA, Von Hundelshausen P and Ley K. Platelet chemokines in vascular disease. *Arterioscler Thromb Vasc Biol* 2008; 28: 1920–1927.
11. Blair P and Flaumenhaft R. Platelet  $\alpha$ -granules: Basic biology and clinical correlates. *Blood Rev* 2009; 23: 177–189.
12. Schmitt A, Guichard J, Massé JM, et al. Of mice and men: Comparison of the ultrastructure of megakaryocytes and platelets. *Exp Hematol* 2001; 29: 1295–1302.
13. Angus (Pitt) DC and van der Poll T. Severe sepsis and septic shock. *N Engl J Med* 2013; 369: 840–851.
14. Lagu T, Rothberg MB, Shieh M-S, et al. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. *Crit Care Med* 2012; 40: 754–761.
15. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29: 1303–1310.
16. Friedman G, Silva E and Vincent JL. Has the mortality of septic shock changed with time? *Crit Care Med* 1998; 26: 2078–2086.
17. Claushuis TAM, van Vught LA, Scicluna BP, et al. Thrombocytopenia is associated with a dysregulated host response in critically ill sepsis patients. *Blood* 2016; 127: blood-2015-11-680744.
18. Foley JH and Conway EM. Cross talk pathways between coagulation and inflammation. *Circ Res* 2016; 118: 1392–1408.
19. Levi M and Van Der Poll T. Inflammation and coagulation. *Crit Care Med* 2010; 38: S26–S34.
20. de Stoppelaar SF, van 't Veer C, Claushuis TAM, et al. Thrombocytopenia impairs host defense in gram-negative pneumonia-derived sepsis in mice. *Blood* 2014; 124: 3781–3790.
21. Goerge T, Ho-Tin-Noe B, Carbo C, et al. Inflammation induces hemorrhage in thrombocytopenia. *Blood* 2008; 111: 4958–4964.
22. Lopez JA. The platelet glycoprotein Ib-IX complex. *Blood Coagul Fibrinolysis* 1994; 5: 97–119.
23. Li R and Emsley J. The organizing principle of the platelet glycoprotein Ib-IX-V complex. *J Thromb Haemost* 2013; 11: 605–614.
24. Plummer C, Wu H, Kerrigan SW, et al. A serine-rich glycoprotein of *Streptococcus sanguis* mediates adhesion to platelets via GPIb. *Br J Haematol* 2005; 129: 101–109.
25. Hartleib J, Köhler N, Dickinson RB, et al. Protein A is the von Willebrand factor binding protein on *Staphylococcus aureus*. *Blood* 2000; 96: 2149–2156.
26. O'Seaghdha M, Van Schooten CJ, Kerrigan SW, et al. *Staphylococcus aureus* protein A binding to von Willebrand factor A1 domain is mediated by conserved IgG binding regions. *FEBS J* 2006; 273: 4831–4841.
27. Bennett JS. Structure and function of the platelet integrin  $\alpha$ IIB $\beta$ 3. *J Clin Invest* 2005; 115: 3363–3369.
28. Coburn J, Leong JM and Erban JK. Integrin  $\alpha$ IIB $\beta$ 3 mediates binding of the Lyme disease agent *Borrelia burgdorferi* to human platelets. *Proc Natl Acad Sci USA* 1993; 90: 7059–7063.
29. Arciola CR, Campoccia D, Gamberini S, et al. Presence of fibrinogen-binding adhesin gene in *Staphylococcus epidermidis* isolates from central venous catheters-associated and orthopaedic implant-associated infections. *Biomaterials* 2004; 25: 4825–4829.
30. Brennan MP, Loughman A, Devocelle M, et al. Elucidating the role of *Staphylococcus epidermidis* serine-aspartate repeat protein G in platelet activation. *J Thromb Haemost* 2009; 7: 1364–1372.
31. Siboo IR, Cheung AL, Bayer AS, et al. Clumping factor A mediates binding of *Staphylococcus aureus* to human platelets. *Infect Immun* 2001; 69: 3120–3127.
32. O'Brien L, Kerrigan SW, Kaw G, et al. Multiple mechanisms for the activation of human platelet aggregation by *Staphylococcus aureus*: Roles for the clumping factors ClfA and ClfB, the serine-aspartate repeat protein SdrE and protein A. *Mol Microbiol* 2002; 44: 1033–1044.
33. Kawai T and Akira S. The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. *Nat Immunol* 2010; 11: 373–384.
34. Cognasse F, Hamzeh H, Chavarin P, et al. Evidence of Toll-like receptor molecules on human platelets. *Immunol Cell Biol* 2005; 83: 196–198.
35. Andonegui G, Kerfoot SM, McNagny K, et al. Platelets express functional Toll-like receptor-4. *Blood* 2005; 106: 2417–2423.
36. Keane C, Tilley D, Cunningham A, et al. Invasive *Streptococcus pneumoniae* trigger platelet activation via Toll-like receptor 2. *J Thromb Haemost* 2010; 8: 2757–2765.
37. Cognasse F, Hamzeh-Cognasse H, Lafarge S, et al. Toll-like receptor 4 ligand can differentially modulate the release of cytokines by human platelets. *Br J Haematol* 2008; 141: 84–91.
38. Ståhl AL, Svensson M, Mörgelin M, et al. Lipopolysaccharide from enterohemorrhagic *Escherichia coli* binds to platelets through TLR4 and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. *Blood* 2006; 108: 167–176.
39. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007; 13: 463–469.
40. von Brühl M-L, Stark K, Steinhart A, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med* 2012; 209: 819–835.
41. Jiménez-Alcázar M, Rangaswamy C, Panda R, et al. Host DNases prevent vascular occlusion by neutrophil extracellular traps. *Science* 2017; 358: 1202–1206.
42. Nieswandt B, Brakebusch C, Bergmeier W, et al. Glycoprotein VI but not  $\alpha$ 2 $\beta$ 1 integrin is essential for platelet interaction with collagen. *EMBO J* 2001; 20: 2120–2130.
43. Nieswandt B and Watson SP. Platelet-collagen interaction: Is GPVI the central receptor? *Blood* 2003; 102: 449–461.
44. Suzuki-Inoue K, Fuller GLJ, García A, et al. A novel Syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2. *Blood* 2006; 107: 542–549.
45. Watson SP, Herbert JMJ and Pollitt AY. GPVI and CLEC-2 in hemostasis and vascular integrity. *J Thromb Haemost* 2010; 8: 1456–1467.
46. Nieswandt B, Schulte V, Bergmeier W, et al. Long-term antithrombotic protection by in vivo depletion of platelet glycoprotein VI in mice. *J Exp Med* 2001; 193: 459–469.
47. Massberg S, Gawaz M, Grüner S, et al. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. *J Exp Med* 2003; 197: 41–49.
48. May F, Hagedorn I, Pleines I, et al. CLEC-2 is an essential platelet activating receptor in hemostasis and thrombosis. *Blood* 2009; 114: 3464–3473.
49. Boulaftali Y, Hess PR, Getz TM, et al. Platelet ITAM signaling is critical for vascular integrity in inflammation. *J Clin Invest* 2013; 123: 908–916.
50. Gros A, Syvannarath V, Lamrani L, et al. Single platelets seal neutrophil-induced vascular breaches via GPVI during immune-complex-mediated inflammation in mice. *Blood* 2015; 126: 1017–1027.



51. Lowe KL, Finney BA, Deppermann C, et al. Podoplanin and CLEC-2 drive cerebrovascular patterning and integrity during development. *Blood* 2015; 125: 3769–3777.
52. Bertozzi CC, Schmaier AA, Mericko P, et al. Platelets regulate lymphatic vascular development through CLEC-2-SLP-76 signaling. *Blood* 2010; 116: 661–670.
53. Hitchcock JR, Cook CN, Bobat S, et al. Inflammation drives thrombosis after Salmonella infection via CLEC-2 on platelets. *J Clin Invest* 2015; 125: 4429–4446.
54. Rayes J, Lax S, Wichaiyo S, et al. The podoplanin-CLEC-2 axis inhibits inflammation in sepsis. *Nat Commun* 2017; 8: 1: 2239.
55. Powers ME, Becker REN, Sailer A, et al. Synergistic action of *Staphylococcus aureus*  $\alpha$ -toxin on platelets and myeloid lineage cells contributes to lethal sepsis. *Cell Host Microbe* 2015; 17: 775–787.
56. Bender M, Hofmann S, Stegner D, et al. Differentially regulated GPVI ectodomain shedding by multiple platelet-expressed proteinases. *Blood* 2010; 116: 3347–3355.
57. Claushuis TAM, de Vos AF, Nieswandt B, et al. Platelet glycoprotein VI aids in local immunity during gram-negative pneumonia-derived sepsis. *Blood* 2017; 131: 864–876.
58. Montague SJ, Delierneux C, Lecut C, et al. Soluble GPVI is elevated in injured patients: Shedding is mediated by fibrin activation of GPVI. *Blood Adv* 2018; 2: 240–251.
59. Boilard E, Nigrovic PA, Larabee K, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* 2010; 327: 580–583.
60. Gitz E, Pollitt AY, Gitz-Francois JJ, et al. CLEC-2 expression is maintained on activated platelets and on platelet microparticles. *Blood* 2014; 124: 2262–2270.
61. Blair P and Flaumenhaft R. Platelet alpha-granules: Basic biology and clinical correlates. *Blood Rev* 2009; 23: 177–189.
62. Frojmovic MM and Milton JG. Human platelet size, shape, and related functions in health and disease. *Physiol Rev* 1982; 62: 185–261.
63. Stegner D, Deppermann C, Kraft P, et al. Munc13-4 mediated secretion is essential for infarct progression but not intracranial haemostasis in acute stroke. *J Thromb Haemost* 2013; 11: 1430–1433.
64. Deppermann C, Cherpokova D, Nurden P, et al. Gray platelet syndrome and defective thrombo-inflammation in Nbeal2-deficient mice. *J Clin Invest* 2013; 123: 3331–3342.
65. May AE, Seizer P and Gawaz M. Platelets: Inflammatory firebugs of vascular walls. *Arterioscler Thromb Vasc Biol* 2008; 28: s5–s10.
66. Braun OÖ, Slotta JE, Menger MD, et al. Primary and secondary capture of platelets onto inflamed femoral artery endothelium is dependent on P-selectin and PSGL-1. *Eur J Pharmacol* 2008; 592: 128–132.
67. Zarbock A, Singbartl K and Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 2006; 116: 3211–3219.
68. Walz A, Dewald B, Tschanner V, et al. Nap-2, Platelet Basic Protein, Connective Platelet Factor 4 on human neutrophils. *J Exp Med* 1989; 170: 1745–1750.
69. Schenk BI, Petersen F, Flad H-D, et al. Platelet-derived chemokines CXC chemokine ligand (CXCL)7, connective tissue-activating peptide III, and CXCL4 differentially affect and cross-regulate neutrophil adhesion and transendothelial migration. *J Immunol* 2002; 169: 2602–2610.
70. Hristov M, Zernecke A, Bidzhekov K, et al. Importance of CXC chemokine receptor 2 in the homing of human peripheral blood endothelial progenitor cells to sites of arterial injury. *Circ Res* 2007; 100: 590–597.
71. Ellis M, Al-Ramadi B, Hedström U, et al. Significance of the CC chemokine RANTES in patients with haematological malignancy: Results from a prospective observational study. *Br J Haematol* 2005; 128: 482–489.
72. Huo Y, Schober A, Forlow SB, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* 2003; 9: 61–67.
73. Von Hundelshausen P, Koenen RR, Sack M, et al. Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. *Blood* 2005; 105: 924–930.
74. Slungaard A. Platelet factor 4: A chemokine enigma. *Int J Biochem Cell Biol* 2005; 37: 1162–1167.
75. Petersen F, Ludwig A, Flad HD, et al. TNF-alpha renders human neutrophils responsive to platelet factor 4. Comparison of PF-4 and IL-8 reveals different activity profiles of the two chemokines. *J Immunol* 1996; 156: 1954–1962.
76. Kasper B, Brandt E, Bulfone-Paus S, et al. Platelet factor 4 (PF-4)-induced neutrophil adhesion is controlled by src-kinases, whereas PF-4-mediated exocytosis requires the additional activation of p38 MAP kinase and phosphatidylinositol 3-kinase. *Blood* 2004; 103: 1602–1610.
77. Kasper B, Brandt E, Brandau S, et al. Platelet factor 4 (CXC chemokine ligand 4) differentially regulates respiratory burst, survival, and cytokine expression of human monocytes by using distinct signaling pathways. *J Immunol* 2007; 179: 2584–2591.
78. Golebiewska EM and Poole AW. Platelet secretion: From haemostasis to wound healing and beyond. *Blood Rev* 2015; 29: 153–162.
79. Scheuerer B, Ernst M, Dürrbaum-Landmann I, et al. The CXC-chemokine platelet factor 4 promotes monocyte survival and induces monocyte differentiation into macrophages. *Blood* 2000; 95: 1158–1166.
80. Fricke I, Mitchell D, Petersen F, et al. Platelet factor 4 in conjunction with IL-4 directs differentiation of human monocytes into specialized antigen-presenting cells. *FASEB J* 2004; 18: 1588–1590.
81. Jouan V, Canron X, Alemany M, et al. Inhibition of in vitro angiogenesis by platelet factor-4-derived peptides and mechanism of action. *Blood* 1999; 94: 984–993.
82. Struyf S, Burdick MD, Proost P, et al. Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet factor-4/CXCL4 and potent inhibitor of angiogenesis. *Circ Res* 2004; 95: 855–857.
83. Lasagni L, Francalanci M, Annunziato F, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. *J Exp Med* 2003; 197: 1537–1549.
84. Youssefian T, Drouin A, Massé JM, et al. Host defense role of platelets: Engulfment of HIV and *Staphylococcus aureus* occurs in a specific subcellular compartment and is enhanced by platelet activation. *Blood* 2002; 99: 4021–4029.
85. Yeaman MR, Tang YIQ, Shen AJ, et al. Purification and in vitro activities of rabbit platelet microbicidal proteins. *Infect Immun* 1997; 65: 1023–1031.
86. Koo SP, Yeaman MR, Nast CC, et al. The cytoplasmic membrane is a primary target for the staphylocidal action of thrombin-induced platelet microbicidal protein. *Infect Immun* 1997; 65: 4795–4800.
87. Dankert J, Van der Werff J, Zaat SAJ, Joldersma W, Klein D, Hess J. Involvement of bactericidal factors from thrombin-stimulated platelets in clearance of adherent viridans streptococci in experimental infective endocarditis. *Infect Immun* 1995; 63: 663–671.
88. Sullam PM, Frank U, Yeaman MR, et al. Effect of thrombocytopenia on the early course of streptococcal endocarditis. *J Infect Dis* 1993; 168: 910–914.
89. Krijgsveld J, Zaat SA, Meeldijk J, et al. Thrombocidins, microbicidal proteins from human blood platelets, are C-terminal deletion products of CXC chemokines. *J Biol Chem* 2000; 275: 20374–20381.



90. McMorran BJ, Marshall VM, De Graaf C, et al. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science* 2009; 323: 797–800.
91. Palankar R, Kohler TP, Krauel K, et al. Platelets kill bacteria by bridging innate and adaptive immunity via PF4 and FcγRIIA. *J Thromb Haemost* 2018; 38: 42–49.
92. Jenne CN and Kubes P. Immune surveillance by the liver. *Nat Immunol* 2013; 14: 996–1006.
93. Endo Y and Nakamura M. The effect of lipopolysaccharide, interleukin-1 and tumour necrosis factor on the hepatic accumulation of 5-hydroxytryptamine and platelets in the mouse. *Br J Pharmacol* 1992; 105: 613–619.
94. Nakamura M, Shibasaki M, Nitta Y, et al. Translocation of platelets into Disse spaces and their entry into hepatocytes in response to lipopolysaccharides, interleukin-1 and tumour necrosis factor: The role of Kupffer cells. *J Hepatol* 1998; 28: 991–999.
95. Lee W-Y, Moriarty TJ, Wong CHY, et al. An intravascular immune response to *Borrelia burgdorferi* involves Kupffer cells and iNKT cells. *Nat Immunol* 2010; 11: 295–302.
96. Zeng Z, Surewaard BGJ, Wong CHY, et al. CRiG Functions as a macrophage pattern recognition receptor to directly bind and capture blood-borne gram-positive bacteria. *Cell Host Microbe* 2016; 20: 99–106.
97. Surewaard BGJ, Deniset JF, Zemp FJ, et al. Identification and treatment of the *Staphylococcus aureus* reservoir in vivo. *J Exp Med* 2016; jem.20160334.
98. Wong CHY, Jenne CN, Petri B, et al. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. *Nat Immunol* 2013; 14: 785–792.
99. Verschoor A, Neuenhahn M, Navarini AA, et al. A platelet-mediated system for shuttling blood-borne bacteria to CD8α<sup>+</sup> dendritic cells depends on glycoprotein GPIb and complement C3. *Nat Immunol* 2011; 12: 1194–1201.
100. Broadley SP, Plaumann A, Coletti R, et al. Dual-track clearance of circulating bacteria balances rapid restoration of blood sterility with induction of adaptive immunity. *Cell Host Microbe* 2016; 20: 36–48.
101. Gawaz M, Fateh-Moghadam S, Pilz G, et al. Platelet activation and interaction with leucocytes in patients with sepsis or multiple organ failure. *Eur J Clin Invest* 1995; 25: 843–851.
102. Page C and Pitchford S. Neutrophil and platelet complexes and their relevance to neutrophil recruitment and activation. *Int Immunopharmacol* 2013; 17: 1176–1184.
103. Htun P, Fateh-Moghadam S, Tomandl B, et al. Course of platelet activation and platelet-leukocyte interaction in cerebrovascular ischemia. *Stroke* 2006; 37: 2283–2287.
104. Moore KL, Varki A and McEver RG. GMP-140 binds to a glycoprotein receptor on human neutrophils: Evidence for a lectin-like interaction. *J Cell Biol* 1991; 112: 491–499.
105. Larsen E, Palabrica T, Sajer S, et al. PADGEM-dependent adhesion of platelets to monocytes and neutrophils is mediated by a lineage-specific carbohydrate, LNF III (CD15). *Cell* 1990; 63: 467–474.
106. Mayadas TN, Johnson RC, Rayburn H, et al. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell* 1993; 74: 541–554.
107. Ley K, Bullard DC, Arbonés ML, et al. Sequential contribution of L- and P-selectin to leukocyte rolling in vivo. *J Exp Med* 1995; 181: 669–675.
108. De Sanctis GT, Wolyniec WW, Green FH, et al. Reduction of allergic airway responses in P-selectin-deficient mice. *J Appl Physiol* 1997; 83: 681–687.
109. Mulligan MS, Polley MJ, Bayer RJ, et al. Neutrophil-dependent acute lung injury. Requirement for P-selectin (GMP-140). *J Clin Invest* 1992; 90: 1600–1607.
110. Kornerup KN, Salmon GP, Pitchford SC, et al. Circulating platelet-neutrophil complexes are important for subsequent neutrophil activation and migration. *J Appl Physiol* 2010; 109: 758–767.
111. Kuligowski MP, Kitching AR and Hickey MJ. Leukocyte recruitment to the inflamed glomerulus: A critical role for platelet-derived P-selectin in the absence of rolling. *J Immunol* 2006; 176: 6991–6999.
112. Singbartl K, Forlow SB and Ley K. Platelet, but not endothelial, P-selectin is critical for neutrophil-mediated acute postischemic renal failure. *FASEB J* 2001; 15: 2337–2344.
113. Sreeramkumar V, Adrover J, Ballesteros I, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science* (80-) 2014; 346: 1235–1238.
114. Langer HF, Choi EY, Zhou H, et al. Platelets contribute to the pathogenesis of experimental autoimmune encephalomyelitis. *Circ Res* 2012; 110: 1202–1210.
115. Frenette PS, Denis CV, Weiss L, et al. P-selectin glycoprotein ligand 1 (Psgl-1) is expressed on platelets and can mediate platelet–endothelial interactions in vivo. *J Exp Med* 2000; 191: 1413–1422.
116. Frenette PS, Johnson RC, Hynes RO, et al. Platelets roll on stimulated endothelium in vivo: An interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci USA* 1995; 92: 7450–7454.
117. Romo GM, Dong JF, Schade AJ, et al. The glycoprotein Ib-IX-v complex is a platelet counterreceptor for P-selectin. *Blood* 1999; 90: 803–813.
118. Dole VS, Bergmeier W, Mitchell HA, et al. Activated platelets induce Weibel-Palade-body secretion and leukocyte rolling in vivo: Role of P-selectin. *Blood* 2005; 106: 2334–2339.
119. Evangelista V, Pamuklar Z, Piccoli A, et al. Src family kinases mediate neutrophil adhesion to adherent platelets. *Blood* 2007; 109: 2461–2469.
120. Simon DI, Chen Z, Xu H, et al. Platelet glycoprotein Ibα is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J Exp Med* 2000; 192: 193–204.
121. Diacovo TG, DeFougerolles AR, Bainton DF, et al. A functional integrin ligand on the surface of platelets: Intercellular adhesion molecule-2. *J Clin Invest* 1994; 94: 1243–1251.
122. Kuijper PH, Gallardo Torres HI, Lammers JW, et al. Platelet associated fibrinogen and ICAM-2 induce firm adhesion of neutrophils under flow conditions. *Thromb Haemost* 1998; 80: 443–448.
123. Nurden AT and Nurden P. Congenital platelet disorders and understanding of platelet function. *Br J Haematol* 2014; 165: 165–178.
124. Weber C and Springer TA. Neutrophil accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to αIIbβ3 and stimulated by platelet-activating factor. *J Clin Invest* 1997; 100: 2085–2093.
125. Henn V, Slupsky JR, Gräfe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* 1998; 391: 591–594.
126. Li G, Sanders JM, Bevard MH, et al. CD40 ligand promotes Mac-1 expression, leukocyte recruitment, and neointima formation after vascular injury. *Am J Pathol* 2008; 172: 1141–1152.
127. Büchner K, Henn V, Gräfe M, et al. CD40 ligand is selectively expressed on CD4<sup>+</sup> T cells and platelets: Implications for CD40-CD40L signalling in atherosclerosis. *J Pathol* 2003; 201: 288–295.
128. Peters MJ, Dixon G, Kotowicz KT, et al. Circulating platelet-neutrophil complexes represent a subpopulation of activated neutrophils primed for adhesion, phagocytosis and intracellular killing. *Br J Haematol* 1999; 106: 391–399.
129. Nagata K, Tsuji T, Todoroki N, et al. Activated platelets induce superoxide anion release by monocytes and neutrophils through P-selectin (CD62). *J Immunol* 1993; 151: 3267–3273.
130. Bengtsson T, Zalavary S, Stendahl O, et al. Release of oxygen metabolites from chemoattractant-stimulated neutrophils is

- inhibited by resting platelets: Role of extracellular adenosine and actin polymerization. *Blood* 1996; 87: 4411–4423.
131. Brinkmann V. Neutrophil extracellular traps kill bacteria. *Science* (80.) 2004; 303: 1532–1535.
  132. Carestia A, Kaufman T and Schattner M. Platelets: New bricks in the building of neutrophil extracellular traps. *Front Immunol* 2016; 7: 271.
  133. Yipp BG, Petri B, Salina D, et al. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat Med* 2012; 18: 1386–1393.
  134. Malawista SE, Van Blaricom G and Breitenstein MG. Cryopreservable neutrophil surrogates. Stored cytoplasts from human polymorphonuclear leukocytes retain chemotactic, phagocytic, and microbicidal function. *J Clin Invest* 1989; 83: 728–732.
  135. McDonald B, Urrutia R, Yipp BG, et al. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* 2012; 12: 324–333.
  136. Kraemer BF, Campbell RA, Schwertz H, et al. Novel anti-bacterial activities of  $\beta$ -defensin 1 in human platelets: Suppression of pathogen growth and signaling of neutrophil extracellular trap formation. *PLoS Pathog* 2011; 7: e1002355.
  137. Caudrillier A, Kessenbrock K, Gilliss BM, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* 2012; 122: 2661–2671.
  138. Rossaint J, Herter JM, Van Aken H, et al. Synchronized integrin engagement and chemokine activation is crucial in neutrophil extracellular trap-mediated sterile inflammation. *Blood* 2014; 123: 2573–2584.
  139. Gaertner F, Ahmad Z, Rosenberger G, et al. Migrating platelets are mechano-scavengers that collect and bundle bacteria. *Cell* 2017; 171: 1368–1382. (e23).
  140. Peerschke EI, Yin W and Ghebrehiwet B. Complement activation on platelets: Implications for vascular inflammation and thrombosis. *Mol Immunol* 2010; 47: 2170–2175.
  141. Peerschke EIB, Murphy TK and Ghebrehiwet B. Activation-dependent surface expression of gC1qR / p33 on human blood platelets. *Thromb Haemost* 2003; 89: 331–339.
  142. Ghebrehiwet B, Lim BL, Peerschke EIB, et al. Isolation, cDNA cloning, and overexpression of a 33-kD cell surface glycoprotein that binds to the globular “heads” of C1q. *J Exp Med* 1994; 179: 1809–1821.
  143. Ghebrehiwet B, Lim BL, Kumar R, et al. gC1q-R/p33, a member of a new class of multifunctional and multicompartamental cellular proteins, is involved in inflammation and infection. *Immunol Rev* 2001; 180: 65–77.
  144. Nguyen T, Ghebrehiwet B and Ellinor IB. *Staphylococcus aureus* Protein A recognizes platelet gC1qR / p33: A novel mechanism for staphylococcal interactions with platelets. *Infect Immun* 2000; 68: 2061–2068.
  145. Hamad OA, Ekdahl KN, Nilsson PH, et al. Complement activation triggered by chondroitin sulfate released by thrombin receptor-activated platelets. *J Thromb Haemost* 2008; 6: 1413–1421.
  146. Ford I, Douglas CW, Heath J, et al. Evidence for the involvement of complement proteins in platelet aggregation by *Streptococcus sanguis* NCTC 7863. *Br J Haematol* 1996; 94: 729–739.
  147. Loughman A, Fitzgerald JR, Brennan MP, et al. Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. *Mol Microbiol* 2005; 57: 804–818.
  148. Hamad OA, Nilsson PH, Wouters D, et al. Complement component C3 binds to activated normal platelets without preceding proteolytic activation and promotes binding to complement receptor 1. *J Immunol* 2010; 184: 2686–2692.
  149. Wiedmer T, Esmon CT and Sims PJ. Complement proteins C5b-9 stimulate procoagulant activity through platelet prothrombinase. *Blood* 1986; 68: 875–880.
  150. Verschoor A and Langer HF. Crosstalk between platelets and the complement system in immune protection and disease. *Thromb Haemost* 2013; 110: 910–919.
  151. Tedesco F, Pausa M, Nardon E, et al. The cytolytically inactive terminal complement complex activates endothelial cells to express adhesion molecules and tissue factor procoagulant activity. *J Exp Med* 1997; 185: 1619–1627.
  152. Morigi M, Galbusera M, Gastoldi S, et al. Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. *J Immunol* 2011; 187: 172–180.
  153. Lambert MP and Gernsheimer TB. Clinical updates in adult immune thrombocytopenia. *Blood* 2017; 129: 2829–2835.
  154. Cohen YC, Djulbegovic B, Shamai-Lubovitz O, et al. The bleeding risk and natural history of idiopathic thrombocytopenic purpura in patients with persistent low platelet counts. *Arch Intern Med* 2000; 160: 1630–1638.
  155. Arnold DM. Platelet count or bleeding as the outcome in ITP trials. *Am J Hematol* 2012; 87: 945–946.
  156. Lax S, Rayes J, Wichaiyo S, et al. Platelet CLEC-2 protects against lung injury via effects of its ligand podoplanin on inflammatory alveolar macrophages in the mouse. *Am J Physiol Lung Cell Mol Physiol* 2017; 44: ajplung.00023.2017.
  157. Rayes J, Jadoui S, Lax S, et al. The contribution of platelet glycoprotein receptors to inflammatory bleeding prevention is stimulus and organ dependent. *Haematologica* 2018; 103: submitted manuscript.
  158. Boulaftali Y, Hess PR, Kahn ML, et al. Platelet Immunoreceptor Tyrosine-Based Activation Motif (ITAM) signaling and vascular integrity. *Circ Res* 2014; 114: 1174–1184.
  159. Deppermann C, Kraft P, Volz J, et al. Platelet secretion is crucial to prevent bleeding in the ischemic brain but not in the inflamed skin or lung in mice. *Blood* 2017; 129: 1702–1707.
  160. Kleinschnitz C, Pozgajova M, Pham M, et al. Targeting platelets in acute experimental stroke: Impact of glycoprotein Ib, VI, and IIb/IIIa blockade on infarct size, functional outcome, and intracranial bleeding. *Circulation* 2007; 115: 2323–2330.
  161. Savage JS, Williams CM, Konopatskaya O, et al. Munc13-4 is critical for thrombosis through regulating release of ADP from platelets. *J Thromb Haemost* 2013; 11: 771–775.
  162. Guerrero JA, Bennett C, van der Weyden L, et al. Gray platelet syndrome: Pro-inflammatory megakaryocytes and  $\alpha$ -granule loss cause myelofibrosis and confer resistance to cancer metastasis in mice. *Blood* 2014; 124: 3624–3635.
  163. Schumacher D, Strilic B, Sivaraj KK, et al. Platelet-derived nucleotides promote tumor-cell transendothelial migration and metastasis via P2Y2 receptor. *Cancer Cell* 2013; 24: 1–8.
  164. Ho-Tin-Noé B, Goerge T, Cifuni SMS, et al. Platelet granule secretion continuously prevents intratumor hemorrhage. *Cancer Res* 2008; 68: 6851–6858.
  165. Higgins SJ, Purcell LA, Silver KL, et al. Dysregulation of angiopoietin-1 plays a mechanistic role in the pathogenesis of cerebral malaria. *Sci Transl Med* 2016; 8: 358ra128.
  166. Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol Neurobiol* 2000; 20: 131–147.
  167. Hillgruber C, Pöppelmann B, Weishaupt C, et al. Blocking neutrophil diapedesis prevents hemorrhage during thrombocytopenia. *J Exp Med* 2015; 212: 1255–1266.
  168. Rayes J and Watson SP. Platelet GPVI repairs its own damage. *Blood* 2015; 126: 933–934.