Platelets in neutrophil recruitment to sites of inflammation

Simon Pitchford[1], **Dingxin Pan**[1,3], and **Heidi C. E. Welch**[2,4]

[1]Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London SE1 9NH, UK

[2]Signalling Programme, Babraham Institute, Cambridge CB22 3AT, United Kingdom

Abstract

Purpose of review—This review describes the essential roles of platelets in neutrophil recruitment from the blood stream into inflamed and infected tissues, with a focus on recent findings.

Recent findings—Platelets are required for the recruitment of neutrophils to sites of inflammation and infection. They fulfil this role largely by enabling contacts of circulating neutrophils with the inflamed blood vessel wall prior to extravasation. Platelets promote both early stages of neutrophil recruitment (tethering, rolling, arrest, firm adhesion) and - as recent work has demonstrated – later stages (intravascular crawling and diapedesis). Recent studies have also begun to identify platelet signaling pathways that can elicit the underlying interactions between platelets, neutrophils and vascular endothelial cells without stimulating concomitant platelet aggregation and thrombus formation. These pathways include Rho-GTPases and Rho-GEFs.

Summary—Recent findings have contributed to our burgeoning understanding of the platelet-dependent mechanisms that control neutrophil recruitment to sites of inflammation and have opened up new avenues of research aimed at increasing our knowledge of these mechanism further. These insights might lead to the development of novel anti-inflammatory drugs that will be useful in a wide range of inflammatory diseases without causing immunodeficiency.

Keywords

Platelets; neutrophils; guanine-nucleotide exchange factors (GEFs); P-Rex1; Vav; P-Selectin Glycoprotein Ligand-1 (PSGL-1); P-selectin

Introduction

Neutrophils are rapidly recruited from the blood stream into inflamed and infected tissues, where they release proinflammatory chemokines and cytokines to attract other inflammatory cells and where they mount effector functions to clear bacterial and fungal infections [1–3]. The importance of neutrophil recruitment and neutrophil effector functions is evident in

[4]Correspondence to: Heidi Welch, Signalling Programme, The Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK. Phone: +44 1223 496 596. Fax: +44 1223 496 063. heidi.welch@babraham.ac.uk.
[3]Present address: Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston MA 02115, USA

Conflicts of interest: The authors have no conflicts of interest to declare.

Human Leukocyte Adhesion Deficiency disorders and in Neutrophil Immunodeficiency Syndrome, which are characterized by severe recurrent infections [4, 5]. Thus, neutrophil recruitment is clearly crucial for innate immunity. However, it must be tightly regulated, as excessive recruitment can lead to inflammatory disorders, tissue destruction and loss of organ function [1–3].

Over recent years, platelets have become recognized as essential regulators of the recruitment and effector functions of many types of leukocytes, including neutrophils, under a wide range of inflammatory conditions. Importantly, these pro-inflammatory roles of platelets are distinct from their well-understood role in hemostasis (blood clotting), which is largely unaffected during inflammation. The various roles of platelets during inflammation have been reviewed extensively over the past 18 months [6–11]. Here we focus on neutrophil recruitment.

Studies using methods to deplete platelet levels or to inhibit the interactions of platelets with circulating neutrophils and with vascular endothelial cells have shown that platelets are required for neutrophil recruitment in many acute and chronic inflammatory situations, including acute septic, aseptic or allergic pulmonary inflammation [12–19], peritonitis [15], pancreatitis [20], atherosclerosis [21], rheumatoid arthritis [22] and encephalomyelitis (multiple sclerosis) [23].

Platelet-dependent cell interactions in neutrophil recruitment

Neutrophil recruitment from the circulation into inflamed and infected tissues proceeds in well-defined steps. Neutrophils are initially captured from the fast-flowing blood stream in the postcapillary microvasculature by selectin-dependent loose tethering to the endothelial vessel wall, followed by rolling along the endothelium, integrin-dependent arrest and firm adhesion, intravascular crawling and trans-endothelial migration into the inflamed tissue [1]. There is evidence that platelets promote all steps of this process from tethering to transmigration (Figure 1).

Inflammatory stimuli induce the upregulation of adhesion molecules on the surface of platelets and vascular endothelial cells that enables these cells to stick to each other and to neutrophils, thus building physical bridges that vastly increase the efficiency of the tethering, rolling and adhesion of neutrophils on the vascular endothelium. In addition to these physical contacts, platelets stimulate adhesion-molecule dependent signaling in neutrophils and vascular endothelial cells, and secrete soluble factors which further promote neutrophil adhesion.

A highly significant adhesion molecule for platelet-neutrophil interactions is P-selectin on the platelet surface [24] which binds P-Selectin Glycoprotein Ligand 1 (PSGL-1) on the neutrophil surface. The importance of platelet P-selectin was determined by the use of P-selectin deficient mice or blocking antibodies for P-selectin and PSGL-1 [13, 25–32] which showed that this interaction is crucial for the interactions of neutrophils with the vascular endothelium from the earliest stages of recruitment (tethering, slings) through to transmigration (please also see reference [33] for a recent review). Binding of platelet P-

selectin to neutrophil PSGL-1 induces inside-out signaling that causes neutrophil β2 integrins to adopt their open conformation [34–36]. Furthermore, a recent landmark publication showed that platelet P-selectin dependent neutrophil PSGL-1 signaling must be spatially restricted to the neutrophil uropod to ensure the correct localization of the chemokine receptors and integrins that control neutrophil intravascular adhesion and crawling [32].

In addition to platelet P-selectin/neutrophil PSGL-1, various integrin- and glycoprotein dependent interactions contribute to platelet-neutrophil interactions. For example, platelet intercellular adhesion molecule-2 (ICAM-2) binds the neutrophil integrin leukocyte function antigen-1 (LFA-1, CD11a/CD18), platelet glycoprotein GPIb binds the neutrophil integrin Macrophage-1 Antigen (Mac-1, complement receptor 3, CR3, CD11b/CD18), and platelet CD40L interacts with neutrophil CD40, a protein of the TNF-receptor superfamily. Platelets also express PSGL-1 [37], and it seems likely that binding of neutrophil L-selectin to this platelet PSGL-1 also contributes to platelet-neutrophil interactions, but that remains to be investigated. Moreover, platelets and neutrophils can bind indirectly to each other using extracellular matrix proteins as bridges, for example both the platelet glycoprotein integrin $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) and neutrophil Mac-1 can bind to fibrinogen (please see also reference [33] and references therein).

Similarly, platelet attachment to the endothelium (which can be extensive, with platelets effectively "carpeting" the vessel wall) is conferred by endothelial P-selectin (and possibly also E-selectin) to platelet PSGL-1 [37], as well as by glycoprotein and integrin-dependent interactions such as platelet GPIbα or GPIIb/IIIa binding to endothelial von Willebrand factor (vWF) or ICAM-1 [38–40]. Endothelial P-selectin is stored on Weibel-Palade bodies which are acutely upregulated in response to inflammatory mediators such as tumor necrosis factor-α (TNFα), and platelets can also induce this upregulation, by secreting serotonin (5-HT) [41, 42]. A recent report showed that platelet adhesion to the vascular endothelium occurs predominantly at endothelial cell junctions, and that these junctional platelets not only facilitate neutrophil capture in a P-selectin dependent manner, but actually guide rolling and crawling neutrophils to their site of transmigration, which was believed to be largely a matter of chance up to now, with neutrophils happening upon weakened endothelial cell/cell junctions [40].

Among the platelet ligands that contribute to interactions with integrins on neutrophils and vascular endothelial cells are ICAM-2 [43], various junctional adhesion molecules (JAMs) and platelet endothelial cell adhesion molecule-1 (PECAM-1). These platelet JAMs and PECAM-1 are required for neutrophil transendothelial migration, at least in part by modulating the strength of the endothelial cell/cell interactions that govern the barrier function of the vasculature [10, 44, 45].

It should be noted that the types of adhesion molecules that confer physical interactions between platelets, neutrophils and vascular endothelial cells vary between inflamed tissues and organs. For example, neutrophil recruitment to the lung seems to be more dependent on PSGL-1 than on P-selectin, unlike in other tissues, suggesting that PSGL-1 interactions with

other types of selectins, such as L- and E-selectin, may important during pulmonary inflammation [13, 33].

Activated platelets release a host of chemokines that can activate neutrophils, including platelet factor 4 (PF4, CXCL4), RANTES (CCL5), neutrophil-activating peptide-2 (NAP-2, CXCL7) and the CXCR2 ligand platelet basic protein (PBP). Both PF4 and NAP-2 stimulate neutrophil endothelial adhesion, and NAP-2 also stimulates neutrophil chemokinesis, chemotaxis and trans-endothelial migration, which are important during recruitment steps following neutrophil arrest [33, 46]. This ability of platelets to stimulate neutrophil migration is P-selectin dependent and can be induced by the activation of platelets through factors that do not stimulate neutrophil migration directly, such as the chemokines MDC (CCL22) and TARC (CCL17) [21]. Accordingly, platelet-derived RANTES and NAP-2 were shown to be required for neutrophil recruitment during lipopolysaccharide (LPS)-induced acute lung injury (ALI) [47]. NAP-2 derived from thrombi of platelets on the vessel wall was also recently found to induce intravascular neutrophil chemotaxis towards the thrombus during vascular injury [48].

Proinflammatory platelets also induce the release of chemokines from vascular endothelial cells, through the secretion of storage vesicles, including interleukin-8 (IL-8, CXCL8) from Weibel-Palade bodies. Rather than disappearing into the circulation, secreted IL-8 remains immobilized on the endothelial surface and stimulates the GPCR CXCR1 on neutrophils [49]. This leads to the integrin Mac-1 on the neutrophil surface adopting its open, active conformation through inside-out signaling, thus stimulating firm neutrophil adhesion, intravascular crawling and transmigration [50].

Moreover, it has recently been demonstrated that soluble platelet-derived factors can also control neutrophil extravasation at a step after transmigration, at least in the lung. Signaling through the Wnt GPCR signaling pathway inhibits the expression of ICAM-1 and VCAM-1 on the surface of alveolar epithelial cells, and platelets were shown to secrete the Wnt antagonist Dickkopf-1 (Dkk1) to block this pathway, thus allowing neutrophils undergoing diapedesis to adhere to these epithelial cells and be recruited into the lung during ALI [18].

It remains to be seen if similar platelet-dependent pathways operate in other tissues to stimulate neutrophil recruitment steps beyond diapedesis, or whether this is a characteristic feature of lung alveolae, where endothelial and epithelial cells are closely adjacent without much interstitium to separate them. Platelets, or microparticles derived from platelets, can certainly be observed in many inflamed tissues [6, 22, 23, 51], but it remains unclear how they get there. They might be piggy-backing on neutrophils, be flushed passively though a leaky endothelium, or be actively migrating by chemotaxis. Support for the second possibility comes from the finding that platelet microparticles, small enough to pass through endothelial cell/cell junctions, are present in synovial fluid of rheumatoid arthritis patients [22, 52]. Support for the third possibility comes from studies reporting chemotaxis of isolated platelets in response to the stimulation of CXCR4 by stromal-derived factor-1 (SDF-1) in a PI3K-dependent manner [53], and in response to allergen after prior in vivo sensitization of mice with ovalbumin [51]. In general though, the fate of platelet-neutrophil complexes beyond the vasculature and the importance of platelet-derived soluble mediators

in stimulating interstitial neutrophil migration towards sources of inflammation and infection still requires much investigation.

Platelet dependent signalling pathways that control neutrophil recruitment

While the surface molecules through which platelets interact with neutrophils and vascular endothelial cells to mediate neutrophil recruitment are now quite well-understood, the platelet signaling pathways that elicit these interactions without causing concomitant platelet aggregation and blood clotting are still quite unclear.

Platelet P-selectin is stored on the membrane of α-granules under basal conditions. Its upregulation onto the platelet surface is one of the earliest events during neutrophil recruitment and is required for platelet-neutrophil interactions. This upregulation occurs through the degranulation of platelet α-granules in response diverse stimuli, including inflammatory mediators such as chemokines, immunoglobulins and factors derived from bacteria (e.g. LPS, a component of the outer membrane of gram-negative bacteria such as Escherichia coli) [33]. However, degranulation occurs in response to pro-thrombotic platelet agonists such as thrombin, and is thus not a means of generating pro-inflammatory platelets specifically.

Adhesion molecule-dependent pathways are elicited through the physical interactions of platelets with neutrophils and vascular endothelial cells. Platelet PSGL-1 signaling has not been studied yet in detail, but by analogy with PSGL-1 signaling pathways in other cell types [36, 37, 50], we can infer that the engagement of platelet PSGL-1 by neutrophil or endothelial selectins is likely to induce ITAM-dependent signaling through Src, Syk and Btk family protein tyrosine kinases, phosphoinositide 3-kinase (PI3K), phospholipase C and mitogen activated kinases (MAPKs), and that this would result in the upregulation and open conformation of platelet integrins.

Activation of platelet integrins by neutrophil and endothelial integrin ligands induces signal transduction through protein tyrosine kinases, PI3K, Rap1, Rho-GEFs and Rho-GTPases (see below), among other effectors, to stimulate platelet adhesion and spreading [54, 55]. However, as with P-selectin, integrin signaling is not specific to proinflammatory platelets, it also promotes aggregation and thrombus formation [54]. For example, during bleeding, platelets bind to collagen on the extraluminal side of the blood vessel exposed by tissue injury through $\alpha_2\beta_1$ (GP1a/IIa, VLA-2) (and indirectly through GPIIb/IIIa, via von vWF) [56], and during blood clotting they bind to fibrinogen and fibrin via $\alpha_{IIb}\beta_3$ [57]. Thus, the pro-inflammatory versus pro-thrombotic outcome of platelet integrin signaling depends on the type of integrin ligands they are exposed to, as well as on crosstalk with other pathways elicited by the presence of inflammatory stimuli.

It is increasingly becoming apparent that, in addition to adhesion molecule-dependent pathways, toll-like receptor (TLR) and G protein coupled receptor (GPCR) signaling pathways are important for proinflammatory platelet functions [6, 7, 58]. Platelets express various TLRs (including TLR1, 2, 4 and 9) which recognize pathogen-associated molecular patterns (PAMPs) [33]. Signaling through the LPS receptor, TLR4, is emerging as

particularly important for platelet-dependent neutrophil recruitment, as it can induce platelet-neutrophil interactions without causing platelet aggregation (although it increases aggregation induced by other stimuli) [59–61]. In nucleated cells, TLR4 signals through the adaptor protein Myd88, the IRAK kinases, TRAF proteins, Akt and Jnk to induce nuclear events (e.g. NFκB dependent transcription) [62], and at least the cytoplasmic part of this pathway also operates in platelets [63, 64]. However, a recent study using platelet-specific deletion of MyD88 suggested that platelet MyD88 is dispensable for pulmonary neutrophil recruitment and the development inflammation during Klebsiella-induced septic pneumonia [65]. This raises the possibility that TLR4 signaling through Myd88-independent pathways (perhaps cGMP [63]) may be more important during platelet-dependent neutrophil recruitment.

Among platelet GPCR pathways, signaling through thrombin receptors PAR1 and PAR4 is the archetypical pro-thrombotic pathway, where the protease thrombin cleaves the N-terminus off the receptor to generate an active GPCR [66]. Yet, even that pathway has recently been implicated in neutrophil recruitment, as thrombin-dependent cleavage of platelet PAR4 was shown to induce neutrophil recruitment to sites of vascular injury [67]. Furthermore, the PI3K target Akt2 was recently shown to be required for neutrophil-platelet interactions during vascular inflammation. Inhibition or deficiency of Akt2 in platelets was sufficient to impair the interaction of thrombin-stimulated platelets with neutrophils, suggesting that Akt2 is an important effector of thrombin signaling during vascular inflammation [68].

In comparison to thrombin, the GPCR ligand ADP induces a weaker pro-thrombotic signal, and signaling through the P2Y1 ADP-receptor and its downstream target RhoA was recently shown to be required for platelet-neutrophil interactions and for neutrophil recruitment to the lung during allergic inflammation [69].

In addition to adhesion-molecule and receptor-dependent pathways, proinflammatory platelet functions are also known to be enhanced by extracellular acidosis, a characteristic feature of tissue injury, whereas the hemostatic functions of platelets are decreased by acidosis [33, 70]. However, the molecular basis of this preferential stimulation of the inflammatory functions of platelets under low pH conditions is still unknown.

Platelet Rho-GTPases and Rho-GEFs in neutrophil recruitment

The Rho-family small GTPases Rac1 and RhoA [71] are expressed in platelets and control actomyosin cytoskeletal dynamics, and thus platelet morphology, adhesion and degranulation [72–75]. Recent studies using the Rac1 inhibitor NSC23766 showed that Rac1 activity is required for the secretion of RANTES and PF4 from platelets by regulating the degranulation of α-granules, and that these platelet-derived factors are required for neutrophil recruitment to the lung during bacterial infections [16, 17]. Similarly, the importance of platelet RhoA activity was recently demonstrated by the inability of platelets treated with an inhibitor of the RhoA target ROCK to reconstitute neutrophil recruitment in thrombocytopenic mice during allergic pulmonary inflammation [69].

Rho-GTPases are activated by guanine-nucleotide exchange factors (GEFs) [76]. The Rac-GEFs P-Rex1, Vav1 and Vav3 are known to be expressed in platelets and to play minor roles in platelet aggregation and hemostasis [75, 77, 78]. P-Rex1, which is activated by PIP_3 (the lipid second messenger produced by PI3K) and the $G\beta\gamma$ subunits of heterotrimeric G proteins, controls neutrophil recruitment through both neutrophil-intrinsic and –extrinsic mechanism [79–82]. Neutrophil-intrinsic roles include E-selectin and LFA-1-dependent rolling on vascular endothelium and Mac-1-dependent intravascular crawling [81], whereas neutrophil-extrinsic roles comprise the regulation of vascular permeability through E-cadherin endothelial cell junctions [83]. Vav1 and Vav3, which are activated by tyrosine kinases in response to the stimulation of various receptors such as integrins, Fc receptors and GPCRs [84], alone are dispensable for neutrophil recruitment, but do cooperate with P-Rex1 to regulate neutrophil recruitment and effector functions [15, 72, 85]. Neutrophil transmigration and airway infiltration are all-but-lost in $Prex1^{-/-}Vav1^{-/-}$ and $Prex1^{-/-}Vav3^{-/-}$ mice during LPS-induced pulmonary inflammation, due to an impairment in L- and E-selectin dependent neutrophil adhesion to airway postcapillary venules and in ICAM-1 dependent slow rolling [15].

Importantly, this crucial role of the two Rac-GEF families in neutrophil recruitment was dependent on their expression in platelets. Unlike wild-type platelets, $Prex1^{-/-}Vav1^{-/-}$ or $Prex1^{-/-}Vav3^{-/-}$ platelets failed to reconstitute neutrophil recruitment in thrombocytopenic mice, both during acute septic and allergic pulmonary inflammation [15]. The severity of the phenotype was similar to that seen in $Prex1^{-/-}Vav1^{-/-}$ and $Prex1^{-/-}Vav3^{-/-}$ mice, which suggested that the expression of these Rac-GEFs in platelets is more important for neutrophil recruitment than their neutrophil-intrinsic roles. P-selectin levels were normal on the surface of $Prex1^{-/-}Vav1^{-/-}$ and $Prex1^{-/-}Vav3^{-/-}$ platelets, but PSGL-1 levels were reduced, as was the incidence of platelet-neutrophil complexes in the circulation. Thus, the loss of L- and E-selectin dependent neutrophil adhesion to the airway microvasculature seen in $Prex1^{-/-}Vav1^{-/-}$ and $Prex1^{-/-}Vav3^{-/-}$ was likely caused by impaired interactions of neutrophils with these platelets, due to the reduced surface levels of platelet PSGL-1 [15] (Figure 2).

Finally, the importance of platelet P-Rex and Vav Rac-GEFs was not restricted to neutrophil recruitment. The pulmonary recruitment of eosinophils, monocytes and lymphocytes was equally compromised in $P-Rex1^{-/-}Vav1^{-/-}$ and $P-Rex1^{-/-}Vav3^{-/-}$ mice during allergic inflammation in a platelet-dependent manner [15]. Airway inflammation was essentially abolished in these mice, resulting in improved airway responsiveness [15]. Therefore, platelet P-Rex and Vav family Rac-GEFs play widely important roles in leukocyte recruitment to sites of inflammation. This preferential role in proinflammatory rather than hemostatic platelet functions implies possibilities for novel therapeutic approaches in the treatment of inflammatory disorders.

Conclusion

It should be re-iterated that neutrophil recruitment is only one of the many pro-inflammatory functions of platelets. Platelets are also important for neutrophil effector functions such as reactive oxygen species (ROS) formation, phagocytosis and neutrophil-extracellular traps

(NETs) formation, as well as for the recruitment and effector functions of many other types of leukocytes which have recently been reviewed in detail elsewhere [6–9].

The importance of P-selectin and PSGL-1 for platelet-neutrophil interactions during neutrophil recruitment suggests that targeting these molecules might be an effective strategy for novel anti-inflammatory therapies. Accordingly, several drugs that inhibit the synthesis or function of P-selectin or PSGL-1, including small molecule compounds (Bimosiamose) and blocking antibodies (SelK1, SelG1) are in clinical trials for the treatment of inflammatory disorders such as Crohn's disease, COPD, asthma and psoriasis [86]. Furthermore, compounds that inhibit the synthesis of PSGL-1 under inflammatory conditions, rather than affecting expression at resting state are being developed, with the hope that future drugs will target the requisite role of selectins during leukocyte recruitment without affecting the necessary immune-surveillance of the host [87]. Finally, other experimental drugs, such as a non-anticoagulant form of heparin, have recently been reported to disrupt platelet- dependent neutrophil recruitment in animal models [88]. In the future, it may thus possible to use similar therapeutic strategies to specifically target the formation of platelet-dependent inflammatory disorders without affecting the hemostatic functions of platelets.

Acknowledgements

Abbreviations

ALI	acute lung injury
Dkk1	Dickkopf-1
GEF	guanine-nucleotide exchange factor
GPCR	G protein coupled receptor
ICAM	intercellular adhesion molecule
IL-8	interleukin-8
JAMs	junctional adhesion molecules
LAD	leukocyte adhesion deficiency
LFA-1	leukocyte function antigen-1
LPS	lipopolysccharide
Mac-1	Macrophage-1 Antigen
MAPK	mitogen activated kinase

NAP-2	neutrophil-activating peptide-2
PBP	platelet basic protein
PECAM-1	platelet endothelial cell adhesion molecule-1
PI3K	phosphoinositide 3-kinase
PIP$_3$	phosphatidyl-inositol (3,4,5) trisphosphate
PF4	platelet factor 4
P-Rex	PIP$_3$-dependent Rac exchanger
PSGL-1	P-selectin glycoprotein ligand-1
ROCK	Rho-associated protein kinase
RTK	receptor tyrosine kinase
GTPase	guanine-nucleotide binding protein
SDF-1	stromal-derived factor-1
TNFα	tumor necrosis factor-α
TLR	toll-like receptor
vWF	von Willebrand factor

References (recent primary papers annotated)

[1]. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013; 13:159–175. [PubMed: 23435331]

[2]. Nauseef WM, Borregaard N. Neutrophils at work. Nat Immunol. 2014; 15:602–611. [PubMed: 24940954]

[3]. Mocsai A. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. J Exp Med. 2013; 210:1283–1299. [PubMed: 23825232]

[4]. Keszei M, Westerberg LS. Congenital defects in neutrophil dynamics. J Immunol Res. 2014; 2014:303782. [PubMed: 25165726]

[5]. Troeger A, Williams DA. Hematopoietic-specific Rho GTPases Rac2 and RhoH and human blood disorders. Exp Cell Res. 2013; 319:2375–2383. [PubMed: 23850828]

[6]. Jenne CN, Kubes P. Platelets in inflammation and infection. Platelets. 2015; 26:286–292. [PubMed: 25806786]

[7]. Rossaint J, Zarbock A. Platelets in leucocyte recruitment and function. Cardiovasc Res. 2015; 107:386–395. [PubMed: 25712962]

[8]. Thomas MR, Storey RF. The role of platelets in inflammation. Thromb Haemost. 2015; 114:449–458. [PubMed: 26293514]

[9]. Kral JB, Schrottmaier WC, Salzmann M, Assinger A. Platelet Interaction with Innate Immune Cells. Transfus Med Hemotherapy. 2016; 43:78–88.

[10]. Gros A, Ollivier V, Ho-Tin-Noe B. Platelets in inflammation: regulation of leukocyte activities and vascular repair. Front Immunol. 2014; 5:678. [PubMed: 25610439]

[11]. Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. Blood. 2015; 126:582–588. [PubMed: 26109205]

[12]. Pitchford SC, Yano H, Lever R, et al. Platelets are essential for leukocyte recruitment in allergic inflammation. J Allergy Clin Immunol. 2003; 112:109–118. [PubMed: 12847487]

[13]. 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. [PubMed: 20558756]

[14]. Page C, Pitchford S. Platelets and allergic inflammation. Clin Exp Allergy. 2014; 44:901–913. [PubMed: 24708345]

[15]. Pan D, Amison RT, Riffo-Vasquez Y, et al. P-Rex and Vav Rac-GEFs in platelets control leukocyte recruitment to sites of inflammation. Blood. 2015; 125:1146–1158. [PubMed: 25538043] [* This paper revealed that the Rac-GEFs P-Rex1, Vav1 and Vav3 cooperate in neutrophil recruitment by conferring neutrophil intravascular adhesion. P-Rex and Vav deficiency in platelets alone was sufficient to recapitulate the neutrophil recruitment defects seen in P-Rex1/Vav deficient mice, likely by causing a reduction in platelet PSGL-1 surface levels which led to reduced platelet-neutrophil interactions in the circulation.]

[16]. Hwaiz R, Rahman M, Zhang E, Thorlacius H. Platelet secretion of CXCL4 is Rac1-dependent and regulates neutrophil infiltration and tissue damage in septic lung damage. Brit J Pharmacol. 2015; 172:5347–5359. [PubMed: 26478565]

[17]. Hwaiz R, Rahman M, Syk I, et al. Rac1-dependent secretion of platelet-derived CCL5 regulates neutrophil recruitment via activation of alveolar macrophages in septic lung injury. J Leuk Biol. 2015 pii: jlb.4A1214-603R [Epub ahead of print]. [* The two papers here-above and ref. 20 showed that platelets secrete CXCL4 and CCL5 in a Rac1-dependent manner during abdominal sepsis. Inhibition of these chemokines reduced neutrophil recruitment, oedema and tissue damage, likely by preventing the chemokine-stimulated generation of CXCL2 by alveolar macrophages. Patients with acute pancreatitis were shown to have significantly elevated plasma levels of CXCL4.]

[18]. Guo Y, Mishra A, Howland E, et al. Platelet-derived Wnt antagonist Dickkopf-1 is implicated in ICAM-1/VCAM-1-mediated neutrophilic acute lung inflammation. Blood. 2015; 126:2220–2229. [PubMed: 26351298] [** Wnt signaling limits the adhesion of neutrophils to alveolar epithelial cells. This paper identified platelet-derived Dickkopf-1 (Dkk1) as an antagonist that suppresses Wnt signaling in alveolar epithelial cells during acute lung inflammation. A Dkk1-neutralizing antibody reduced neutrophil recruitment during lung injury, suggesting this pathway has potential therapeutic value to limit tissue damage during acute pulmonary inflammation.]

[19]. Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. J Clin Invest. 2006; 116:3211–3219. [PubMed: 17143330]

[20]. Wetterholm E, Linders J, Merza M, et al. Platelet-derived CXCL4 regulates neutrophil infiltration and tissue damage in severe acute pancreatitis. Transl Res. 2016

[21]. 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. [PubMed: 12483207]

[22]. Boilard E, Nigrovic PA, Larabee K, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. Science. 2010; 327:580–583. [PubMed: 20110505]

[23]. Langer HF, Choi EY, Zhou H, et al. Platelets contribute to the pathogenesis of experimental autoimmune encephalomyelitis. Circ Res. 2012; 110:1202–1210. [PubMed: 22456181]

[24]. Larsen E, Celi A, Gilbert GE, et al. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell. 1989; 59:305–312. [PubMed: 2478294]

[25]. 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. [PubMed: 7688665]

[26]. Moore KL, Patel KD, Bruehl RE, et al. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. J Cell Biol. 1995; 128:661–671. [PubMed: 7532174]

[27]. Patel KD, Moore KL, Nollert MU, McEver RP. Neutrophils use both shared and distinct mechanisms to adhere to selectins under static and flow conditions. J Clin Invest. 1995; 96:1887–1896. [PubMed: 7560080]

[28]. Schmidtke DW, Diamond SL. Direct observation of membrane tethers formed during neutrophil attachment to platelets or P-selectin under physiological flow. J Cell Biol. 2000; 149:719–730. [PubMed: 10791984]

[29]. Pitchford SC, Momi S, Giannini S, et al. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. Blood. 2005; 105:2074–2081. [PubMed: 15528309]

[30]. Lam FW, Burns AR, Smith CW, Rumbaut RE. Platelets enhance neutrophil transendothelial migration via P-selectin glycoprotein ligand-1. American journal of physiology Heart Circ Physiol. 2011; 300:H468–475.

[31]. Sundd P, Gutierrez E, Koltsova EK, et al. 'Slings' enable neutrophil rolling at high shear. Nature. 2012; 488:399–403. [PubMed: 22763437]

[32]. Sreeramkumar V, Adrover JM, Ballesteros I, et al. Neutrophils scan for activated platelets to initiate inflammation. Science. 2014; 346:1234–1238. [PubMed: 25477463] [** This paper described that neutrophils bind activated circulating platelets via PSGL-1 at their uropod. Spatially restricted platelet-dependent PSGL-1 signaling was shown to ensure the correct distribution of Mac-1 and IL-8 receptor over the neutrophil surface and was required for neutrophil intravascular crawling.]

[33]. Page C, Pitchford S. Neutrophil and platelet complexes and their relevance to neutrophil recruitment and activation. Int Immunopharmacol. 2013; 17:1176–1184. [PubMed: 23810443]

[34]. Evangelista V, Manarini S, Rotondo S, et al. Platelet/polymorphonuclear leukocyte interaction in dynamic conditions: evidence of adhesion cascade and cross talk between P-selectin and the beta 2 integrin CD11b/CD18. Blood. 1996; 88:4183–4194. [PubMed: 8943853]

[35]. Wang HB, Wang JT, Zhang L, et al. P-selectin primes leukocyte integrin activation during inflammation. Nat Immunol. 2007; 8:882–892. [PubMed: 17632516]

[36]. Zarbock A, Muller H, Kuwano Y, Ley K. PSGL-1-dependent myeloid leukocyte activation. J Leuk Biol. 2009; 86:1119–1124.

[37]. 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. [PubMed: 10770806]

[38]. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial P-selectin. Proc Natl Acad Sci USA. 1995; 92:7450–7454. [PubMed: 7543682]

[39]. Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: evidence for a GPIIbIIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alphavbeta3 integrin, and GPIbalpha. J Exp Med. 1998; 187:329–339. [PubMed: 9449713]

[40]. Zuchtriegel G, Uhl B, Puhr-Westerheide D, et al. Platelets guide leukocytes to their sites of extravasation. PLoS Biol. 2016; 14:e1002459. [PubMed: 27152726] [** This outstanding paper described a previously unrecognized role of platelets in guiding neutrophils to their site of trans-endothelial migration. Platelets were found to adhere preferentially to endothelial cell/cell junctions and to direct rolling and crawling neutrophils to these sites.]

[41]. 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. [PubMed: 15956287]

[42]. Duerschmied D, Suidan GL, Demers M, et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. Blood. 2013; 121:1008–1015. [PubMed: 23243271]

[43]. Diacovo TG, deFougerolles AR, Bainton DF, Springer TA. A functional integrin ligand on the surface of platelets: intercellular adhesion molecule-2. J Clin Invest. 1994; 94:1243–1251. [PubMed: 8083366]

[44]. Woodfin A, Reichel CA, Khandoga A, et al. JAM-A mediates neutrophil transmigration in a stimulus-specific manner in vivo: evidence for sequential roles for JAM-A and PECAM-1 in neutrophil transmigration. Blood. 2007; 110:1848–1856. [PubMed: 17505016]

[45]. Chavakis T, Keiper T, Matz-Westphal R, et al. The junctional adhesion molecule-C promotes neutrophil transendothelial migration in vitro and in vivo. J Biol Chem. 2004; 279:55602–55608. [PubMed: 15485832]

[46]. Walz A, Dewald B, von Tscharner V, Baggiolini M. Effects of the neutrophil-activating peptide NAP-2, platelet basic protein, connective tissue-activating peptide III and platelet factor 4 on human neutrophils. J Exp Med. 1989; 170:1745–1750. [PubMed: 2681518]

[47]. Grommes J, Alard JE, Drechsler M, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. Am J Resp Crit Care Med. 2012; 185:628–636. [PubMed: 22246174]

[48]. Ghasemzadeh M, Kaplan ZS, Alwis I, et al. The CXCR1/2 ligand NAP-2 promotes directed intravascular leukocyte migration through platelet thrombi. Blood. 2013; 121:4555–4566. [PubMed: 23550035]

[49]. Middleton J, Patterson AM, Gardner L, et al. Leukocyte extravasation: chemokine transport and presentation by the endothelium. Blood. 2002; 100:3853–3860. [PubMed: 12433694]

[50]. Mocsai A, Walzog B, Lowell CA. Intracellular signalling during neutrophil recruitment. Cardiovasc Res. 2015; 107:373–385. [PubMed: 25998986]

[51]. Pitchford SC, Momi S, Baglioni S, et al. Allergen induces the migration of platelets to lung tissue in allergic asthma. Am J Resp Crit Care Med. 2008; 177:604–612. [PubMed: 18096710]

[52]. Boilard E, Duchez AC, Brisson A. The diversity of platelet microparticles. Curr Opin Hematol. 2015; 22:437–444. [PubMed: 26214207]

[53]. Kraemer BF, Borst O, Gehring EM, et al. PI3 kinase-dependent stimulation of platelet migration by stromal cell-derived factor 1 (SDF-1). J Mol Med. 2010; 88:1277–1288. [PubMed: 20852838]

[54]. Guidetti GF, Canobbio I, Torti M. PI3K/Akt in platelet integrin signaling and implications in thrombosis. Adv Biol Reg. 2015; 59:36–52.

[55]. McCarty OJ, Zhao Y, Andrew N, et al. Evaluation of the role of platelet integrins in fibronectin-dependent spreading and adhesion. J Thromb Haemost. 2004; 2:1823–1833. [PubMed: 15456495]

[56]. Nuyttens BP, Thijs T, Deckmyn H, Broos K. Platelet adhesion to collagen. Thromb Res. 2011; 127(Suppl 2):S26–29.

[57]. Lisman T, Weeterings C, de Groot PG. Platelet aggregation: involvement of thrombin and fibrin(ogen). Front Biosci. 2005; 10:2504–2517. [PubMed: 15970513]

[58]. Rex S, Beaulieu LM, Perlman DH, et al. Immune versus thrombotic stimulation of platelets differentially regulates signalling pathways, intracellular protein-protein interactions, and alpha-granule release. Thromb Haemost. 2009; 102:97–110. [PubMed: 19572074]

[59]. Aslam R, Speck ER, Kim M, et al. Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo. Blood. 2006; 107:637–641. [PubMed: 16179373]

[60]. Andonegui G, Kerfoot SM, McNagny K, et al. Platelets express functional Toll-like receptor-4. Blood. 2005; 106:2417–2423. [PubMed: 15961512]

[61]. Ward JR, Bingle L, Judge HM, et al. Agonists of toll-like receptor (TLR)2 and TLR4 are unable to modulate platelet activation by adenosine diphosphate and platelet activating factor. Thromb Haemost. 2005; 94:831–838. [PubMed: 16270639]

[62]. Garraud O, Cognasse F. Platelet Toll-like receptor expression: the link between "danger" ligands and inflammation. Inflammation & Allergy Drug Targets. 2010; 9:322–333. [PubMed: 20518724]

[63]. Zhang G, Han J, Welch EJ, et al. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. J Immunol. 2009; 182:7997–8004. [PubMed: 19494325]

[64]. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1beta-rich microparticles. J Immunol. 2011; 186:5489–5496. [PubMed: 21430222]

[65]. de Stoppelaar SF, Claushuis TA, Jansen MP, et al. The role of platelet MyD88 in host response during gram-negative sepsis. J Thromb Haemost. 2015; 13:1709–1720. [PubMed: 26178922] [*Toll-like receptors and their adaptor MyD88 are essential for pathogen recognition and immunity. This paper investigated the role of platelet MyD88 during Klebsiella pneumoniae infections using platelet-specific MyD88 deficiency. Against expectations, platelet MyD88 was found to have no role in the development of this pathology, suggesting that other TLR effectors

may operate in platelets.Despite known literature, platelet MyD88-dependent TLR signaling does not contribute to the host response during gram-negative sepsis.]

[66]. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature. 2000; 407:258–264. [PubMed: 11001069]

[67]. Kaplan ZS, Zarpellon A, Alwis I, et al. Thrombin-dependent intravascular leukocyte trafficking regulated by fibrin and the platelet receptors GPIb and PAR4. Nat Commun. 2015; 6:7835. [PubMed: 26204458] [* Thrombin is a central regulator of hemostasis. This paper demonstrated that thrombin cleavage of its platelet receptor PAR4 promotes neutrophil recruitment to sites of vascular injury. In addition, fibrin was shown to limit this trafficking by forming a physical barrier to neutrophil migration.]

[68]. Li J, Kim K, Hahm E, et al. Neutrophil AKT2 regulates heterotypic cell-cell interactions during vascular inflammation. J Clin Invest. 2014; 124:1483–1496. [PubMed: 24642468] [* This paper identified platelet- (and neutrophil-) derived AKT2 as a regulator of neutrophil-platelet interactions during vascular inflammation. AKT2 activity was found to be elevated in patients with sickle cell disease, and inhibition of AKT2 reduced thromboinflammatory conditions in a mouse model of this disease.]

[69]. Amison RT, Momi S, Morris A, et al. RhoA signaling through platelet P2Y(1) receptor controls leukocyte recruitment in allergic mice. J Allergy Clin Immunol. 2015; 135:528–538. [PubMed: 25445826] [* This paper showed that RhoA mediates purinergic P2Y1 receptor signalling in platelets, a pretreatment with ROCK inhibitor blocked the ability of platelets to reconstitute the pulmonary recruitment of neutrophils in thromobocytopenic mice duing allergic inflammation.]

[70]. Etulain J, Negrotto S, Carestia A, et al. Acidosis downregulates platelet haemostatic functions and promotes neutrophil proinflammatory responses mediated by platelets. Thromb Haemost. 2012; 107:99–110. [PubMed: 22159527]

[71]. Heasman SJ, Ridley AJ. Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat Rev Mol Cell Biol. 2008; 9:690–701. [PubMed: 18719708]

[72]. Baker MJ, Pan D, Welch HC. Small GTPases and their guanine-nucleotide exchange factors and GTPase-activating proteins in neutrophil recruitment. Curr Opin Hematol. 2016; 23:44–54. [PubMed: 26619317]

[73]. Aslan JE, McCarty OJ. Rho GTPases in platelet function. J Thromb Haemost. 2013; 11:35–46. [PubMed: 23121917]

[74]. Pearce AC, McCarty OJ, Calaminus SD, et al. Vav family proteins are required for optimal regulation of PLCgamma2 by integrin alphaIIbbeta3. Biochem J. 2007; 401:753–761. [PubMed: 17054426]

[75]. Pearce AC, Wilde JI, Doody GM, et al. Vav1, but not Vav2, contributes to platelet aggregation by CRP and thrombin, but neither is required for regulation of phospholipase C. Blood. 2002; 100:3561–3569. [PubMed: 12411320]

[76]. Rossman KL, Der CJ, Sondek J. GEF means go: turning on Rho GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol. 2005; 6:167–180. [PubMed: 15688002]

[77]. Aslan JE, Spencer AM, Loren CP, et al. Characterization of the Rac guanine nucleotide exchange factor P-Rex1 in platelets. J Mol Signal. 2011; 6:11. [PubMed: 21884615]

[78]. Qian F, Le Breton GC, Chen J, et al. Role for the guanine nucleotide exchange factor phosphatidylinositol-3,4,5-trisphosphate-dependent rac exchanger 1 in platelet secretion and aggregation. Arterioscler Thromb Vasc Biol. 2012; 32:768–777. [PubMed: 22207728]

[79]. Welch HC, Condliffe AM, Milne LJ, et al. P-Rex1 regulates neutrophil function. Curr Biol. 2005; 15:1867–1873. [PubMed: 16243035]

[80]. Dong X, Mo Z, Bokoch G, et al. P-Rex1 is a primary Rac2 guanine nucleotide exchange factor in mouse neutrophils. Curr Biol. 2005; 15:1874–1879. [PubMed: 16243036]

[81]. Herter JM, Rossaint J, Block H, et al. Integrin activation by P-Rex1 is required for selectin-mediated slow leukocyte rolling and intravascular crawling. Blood. 2013; 121:2301–2310. [PubMed: 23343834]

[82]. Welch HC. Regulation and function of P-Rex family Rac-GEFs. Small GTPases. 2015; 6:1–11. [PubMed: 25862160]

[83]. Naikawadi RP, Cheng N, Vogel SM, et al. A critical role for phosphatidylinositol (3,4,5)-trisphosphate-dependent Rac exchanger 1 in endothelial junction disruption and vascular hyperpermeability. Circ Res. 2012; 111:1517–1527. [PubMed: 22965143]

[84]. Bustelo XR. Vav family exchange factors: an integrated regulatory and functional view. Small GTPases. 2014; 5:9. [PubMed: 25483299]

[85]. Lawson C, Donald S, Anderson K, et al. P-Rex1 and Vav1 cooperate in the regulation of fMLF-dependent neutrophil responses. J Immunol. 2011; 186:1467–1476. [PubMed: 21178006]

[86]. Bedard PW, Kaila N. Selectin inhibitors: a patent review. Exp Opin Therapeutic Patents. 2010; 20:781–793.

[87]. Kanabar V, Tedaldi L, Jiang J, et al. Base-modified UDP-sugars reduce cell surface levels of P-selectin glycoprotein 1 (PSGL-1) on IL-1beta-stimulated human monocytes. Glycobiology. 2016; [Epub ahead of print]. doi: 10.1093/glycob/cww053 [* This paper described the use of new small-molecule compounds (modified UDP-sugars) for reducing cell surface levels of PSGL-1 on inflammatory monocytes, suggesting that PSGL-1 may be targetable by similar strategies in the future in the treatment of inflammatory disorders.]

[88]. Riffo-Vasquez Y, Somani A, Man F, et al. A Non Anti-coagulant Fraction of Heparin Inhibits Leukocyte Diapedesis into the Lung by an Effect on Platelets. Am J Resp Cell Mol Biol. 2016; [Epub ahead of print]. doi: 10.1165/rcmb.2015-0172OC [* This paper showed that a non-anticoagulant form of heparin can be used to inhibit platelet-dependent neutrophil recruitment to the lung during pulmonary inflammation in mice. It acts by inhibiting diapedesis, likely by reducing the level of P-selectin on the platelet surface.]

Key points

- During inflammation, platelets enable neutrophil capture from the blood stream, rolling and adhesion on the vascular endothelium, intravascular crawling and transmigration.

- Rho-GTPases and Rho-GEFs control the proinflammatory responses of platelets required for neutrophil recruitment during inflammation.

- P-Rex and Vav family Rac-GEFs in platelets are required for the recruitment of neutrophils to the inflamed lung by enabling L- and E-selectin dependent neutrophil adhesion to the pulmonary vascular endothelium.
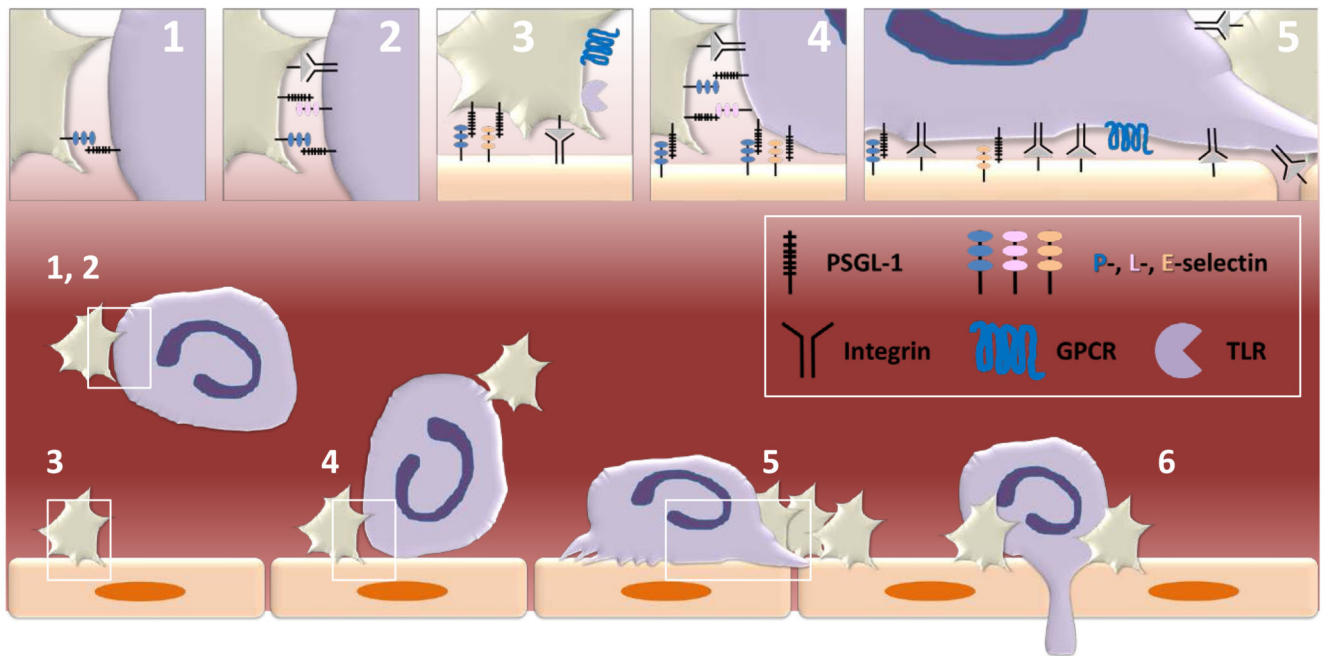
Figure 1. Platelets in neutrophil recruitment

Neutrophil recruitment from the blood stream into inflamed and infected tissues begins with the upregulation of P-selectin on the surface of vascular endothelial cells and circulating platelets in response to inflammatory stimuli such as TNFα and LPS. Binding of platelet P-selectin to neutrophil PSGL-1 confers platelet-neutrophil interactions (**1**) which are strengthened by the engagement pf additional adhesion molecules such as integrins and glycoproteins (**2**). Similar adhesion molecules confer interactions between vascular endothelial cells and platelets, including endothelial P-selectin binding to platelet PSGL-1 (**3**). Physical contacts between all three cell types enable neutrophil tethering and rolling along the vascular endothelium [note that some of the depicted contacts, namely the binding of neutrophil L-selectin and endothelial E-selectin to platelet PSGL-1, are hypothetical; see Figure 2] (**4**). These interactions, together with neutrophil GPCR signaling, induce the upregulation and opening of the neutrophil integrins LFA-1 and Mac-1. Active LFA-1 confers neutrophil arrest and Mac-1 firm adhesion and intravascular crawling (**5**). Signaling through platelet adhesion molecules, TLRs and GPCRs promotes these processes by leading to further cell/cell interactions and to the secretion of soluble factors such as chemokines that activate neutrophils and vascular endothelial cells. Moreover, platelets were recently shown to adhere to the vascular endothelium preferentially at endothelial cell/cell junctions and to actively guide neutrophils to the site of trans-endothelial migration (**6**).
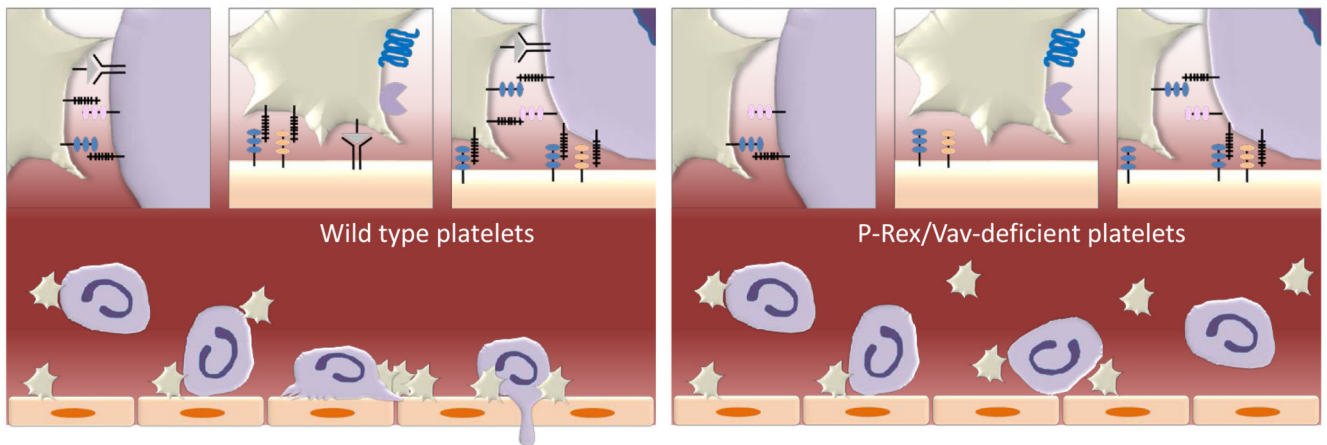
Figure 2. Platelet Rac-GEFs in neutrophil recruitment
Expression of the Rac-GEFs P-Rex1, Vav1 and Vav3 in platelets controls PSGL-1 levels on the platelet surface as well as platelet-neutrophil interactions in the circulation. Prex1/Vav1 or Prex1/Vav3-deficiency in platelets is sufficient to block neutrophil recruitment during septic and allergic pulmonary inflammation by preventing neutrophil adhesion to the airway microvasculature, which is L- and E-selectin dependent. Mechanistically, the lack of PSGL-1 on the surface of Prex1/Vav-deficient platelets could cause the loss of L- and E-selectin dependent neutrophil adhesion if, for example, PSGL-1 on the platelet surface were a ligand for neutrophil L-selectin and endothelial E-selectin in the airway vasculature. A lack of these PSGL-1/selectin interactions would limit further cell/cell contacts by preventing integrin upregulation and activation. Importantly, despite the key roles of platelet P-Rex1 and Vav Rac-GEFs in neutrophil recruitment, Prex1/Vav-deficient platelets undergo normal aggregation in response to thrombin or ADP. Hence, P-Rex1 and Vav Rac-GEFs seem to preferentially control pro-inflammatory rather than pro-thrombotic platelet functions.