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SPOTLIGHT REVIEW

Platelets in leucocyte recruitment and function

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Abstract Platelets have a longstanding recognition as an essential cellular component of the coagulation system. However, substantial research over the last decade has added another important aspect to platelet function in that they are also an integral part of the innate immune system. Complex organisms are facing a constant threat of infections by invading pathogens, and they have developed a sophisticated and elegant measure to combat this threat, namely the immune system. Leucocyte recruitment to sites of infections is an essential step at the forefront of the immune response. Platelets have been shown to be involved in several steps of this process and they are an integrated connecting element among haemostasis, host defence, and additional immunological functions (e.g. neutrophil extracellular traps formation). However, the immune system also requires a tight regulation, as an overshooting immune response carries the risk of harming the host itself. This review aims at highlighting the unique features and molecular mechanisms that allow for the interactions of platelets and leucocytes and the regulation of this process. Furthermore, this article identifies the functional relevance of these events for the immune response.

Keywords Platelets • Leucocytes • Inflammation • Neutrophil Extracellular Traps • Neutrophil Recruitment

This article is part of the Spotlight Issue on Leucocyte trafficking across the vessel wall.

1. Introduction

Leucocyte recruitment is required for an adequate innate immune response.[1,2] In the early phase of the innate immune response, neutrophils are the most abundant leucocyte subset that is recruited to the site of inflammation. Neutrophils are required for effective control of invading pathogens and defects in neutropil recruitment may lead to severe immune deficiency.[3] Nowadays, platelets play a well-recognized role in leucocyte recruitment and research over the past decades revealed many features of immune-modulatory characteristics of platelets.[4] Platelets possess a broad inventory of major receptors and adhesion molecules which enable them to interact with immune cells and also with circulating pathogens.[5] Activated platelets have been shown to directly interact with leucocytes under inflammatory conditions.[6–10] This interaction may lead to the amplification of leucocyte recruitment to the site of inflammation.[11] In addition to the amplification of leucocyte recruitment, the interaction of platelets with leucocytes is also essential for the execution of other immunological task by leucocytes. In that context, platelets have recently been shown to be essential for the formation of neutrophil extracellular traps (NETs) by neutrophils, which are recognized as an effective measure by which neutrophils ensnare and kill circulating bacteria.[8–10,12,13]

In addition to these immunological functions, platelets are also able to sense and to interact with a variety of pathogens directly.[5] In a current study, Wong *et al.*[14] demonstrated that platelets in the liver microcirculation function as an immune surveillance element. Under physiological conditions, platelets scan the vasculature by 'touch-and-go' maneuvers in which they form transient interactions with sinusoidal Kupffer cells by binding of the platelet receptor GPIb to vWF on Kupffer cells. During endotoxaemia, circulating pathogens may be caught by Kupffer cells. In this case, platelets interacting with Kupffer cells become stable, get activated, and eventually initiate the recruitment of neutrophils to successfully combat invading pathogens. Thus, platelets act as sentinels of the immune system by detecting circulating pathogens in collaboration with Kupffer cells. However, it is still unknown if other cell types besides Kupffer cells are also under constant platelet surveillance for presented pathogens, but this is certainly worth to be investigated.

In this review, we aim at presenting the key features that enable platelets to interact with leucocytes, in particular neutrophils, and modulate the immune response. This report may broaden the view on platelets and demonstrate that their role in the immune system is as critical as the role of platelets for primary haemostasis.

2. Platelets: small size, big functions

Platelets are small, anucleated cytoplasmic cell bodies that are present in high numbers in the systemic circulation. They are derived from megakaryocytes and are essentially cellular fragments of these bone-marrow

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resident cells. Platelets are mainly recognized for their importance in primary haemostasis. They may attach to exposed collagen fibres or other components of the sub-endothelial matrix which are normally hidden beyond the endothelium separating them from the blood flow. The formation of stable bonds between platelet-adhesion receptors and collagen induces the activation of platelets and the release of highly active pro-coagulatory mediators (e.g. ADP, thrombin, and prostaglandins). Eventually, this process causes the formation of a thrombus, which also incorporates leucocytes and red blood cells.[15,16] However, traumatic tissue injury does not only carry the threat of blood loss and requires rapid thrombus formation, but also implies that exogenous pathogens, e.g. bacteria, may enter the deeper tissue and eventually the circulation, which leaves the organism vulnerable to local and/or systemic infections. Thus, it is not entirely surprising that the evolution has equipped platelets with immunological functions. In fact, a common cell type known as 'haematocytes' abundant in invertebrates and very early vertebrates was responsible for a broad range of haemostatic and immunological functions.[5,17,18] As a general principle of evolution, diversity of cell types increases with higher orders of organisms. Eventually in mammals, very specialized cells types, e.g. monocytes, neutrophils, lymphocytes, and platelets, have evolved and each developed highly sophisticated cellular functions.

Platelets are well equipped with a broad range of cell-surface receptors and adhesion molecules and, in addition, hold different immune modulatory mediators in their internal storage vesicles (α-granules, dense granules, and lysosome granules).[19] These reservoirs may be released upon activation. The ability of platelets to actively participate in the regulation of immune processes is closely tied to their unique structural characteristics. Platelets are present in the circulation virtually everywhere in large amounts. Thus, it is not surprising that the organism uses platelets as sentinel cells that are able to quickly report any suspicious discoveries back to the immune system itself.

2.1 Platelet activation

Platelets may be activated in various ways, including binding to exposed sub-endothelial structures as well as activation by soluble mediators, e.g. thrombin.[20,21] Upon activation, intracellular signalling cascades in platelets are initiated and these result in cytoskeletal rearrangement and shape change, activation of surface-adhesion molecules, and degranulation. Platelets hold a very divergent stock of inflammatory and coagulatory mediators in their granules, including potent cytokines and chemokines. In fact, the repertoire of mediators enables platelets to fulfil their central roles in many haemostatic pathways, including coagulation and inflammation. Current research indicates that the platelet response, i.e. the activation of surface-adhesion molecule subsets and release of mediators, may actually not be universal, but stimulus-dependent.[22–24] This phenomenon was, however, questioned by the notion that the actual proteomic content of the individual platelets granules does not differ too much from other granules in the same platelet at all, raising the question of how platelets are capable of producing a stimulus-specific mediator release and this remains the focus of ongoing research.[25]

Beyond activation by soluble mediators or binding to adhesion molecules, platelets may also directly interact with circulating pathogens. Platelets have been shown to selectively take up the intracellular pathogen Listeria monocytogenes. This process is followed by adhesion and specific bacterial delivery to $CD8\alpha^+$ dendritic cells, which initiates the further adaptive immune response.[26] Similarly, platelets are required for cytotoxic T cells and viral clearance in lymphocytic choriomeningitis virus (LCMV) infection.[27,28]

To precisely understand the biological role of platelets in the formation of the immune response, an emphasis on the different platelet receptors and surface-adhesion molecules is crucial.

2.2 Platelet-adhesion molecules

2.2.1 Integrins and glycoproteins

Integrins are cell surface-adhesion molecules found on a variety of cell types. They classically mediate the interaction between cells and the extracellular matrix and different cells.[29] Integrins are expressed on platelets and may represent the most important class of adhesion molecules on these cells. Integrins are obligate hetero-dimeric transmembrane proteins being composed of an α-subunit and a β-subunit. Beyond bond formation with their respective ligands, integrins may also transduce signals into the cell.[30] The activation of integrins is defined by their conformational state.[31] Under resting conditions, integrins reside in their low-affinity state, while they switch to an open conformation with high ligand-binding affinity upon activation. Depending on the integrin, one or more transitional conformations exist with intermediate ligand-binding affinity. Integrins are very abundantly expressed on platelets, predominantly β_1- and β_3-integrins. These integrins include $\alpha_5\beta_1$ (VLA-5), $\alpha_6\beta_1$ (VLA-6), $\alpha_2\beta_1$ (VLA-2, GPIa/IIa), and $\alpha_{IIb}\beta_3$ (GPIIb/IIIa).[32,33] Integrins on platelets have diverse functions and are involved in the interaction of platelets with leucocytes, endothelial cells, and the extracellular matrix.[32,33]

Numerous glycoprotein complexes are expressed on platelets including the glycoprotein (GP) Ib–V–IX complex. This complex is the main receptor for binding to the von Willebrand factor (vWF).[34] The GPIb–V–IX–vWF interaction classically mediates the initial contact of platelets with exposed sub-endothelial structures. Furthermore, the glycoproteins GPVI, responsible for platelet binding to collagen, and GPIIb/IIIa (integrin $\alpha_{IIb}\beta_3$), which may bind vWF, fibronectin, or retronectin, are abundantly expressed on platelets.[33,35] GPIIb/IIIa is the most abundant adhesion molecule on platelets. When activated by GPIb or GPCRs, the molecule binds to immobilized and soluble ligands, e.g. fibrinogen, vitronectin, fibronectin, vWF, or thrombospondin.[35] The activation of platelets causes an up-regulation of glycoprotein expression on the cell surface and the activation of these molecules.

Platelet integrins and glycoproteins have been implicated in a number of inflammatory processes. The role of platelet integrins during the contact of platelets with ligands in the sub-endothelial matrix at vascular lesion sites has been extensively investigated. It has been shown *in vitro* that platelet β_3-integrins initiate the firm arrest of platelets to inflamed endothelial cells under static conditions.[36,37] However, their contribution to the interaction of platelets with inflamed vascular endothelium *in vivo* is still an active field of research. It was demonstrated that both the administration of an antibody against the platelet integrin $\alpha_{IIb}\beta_3$ as well as genetic ablation of this integrin decreases firm adhesion of platelets to inflamed vascular endothelial cells *in vivo*.[38] Platelet integrins also play an important role for the formation of platelet–leucocyte complexes during inflammatory processes. The integrin $\alpha_{IIb}\beta_3$ is also required for the formation of stable complexes by binding to Mac-1 on neutrophils via fibrinogen (*Figure 1*).[35] In addition to the mechanical stabilization of the complex, this interaction also induces outside-in signalling in neutrophils inducing important immunological functions, e.g. NET formation and leucocyte recruitment.[8,12]

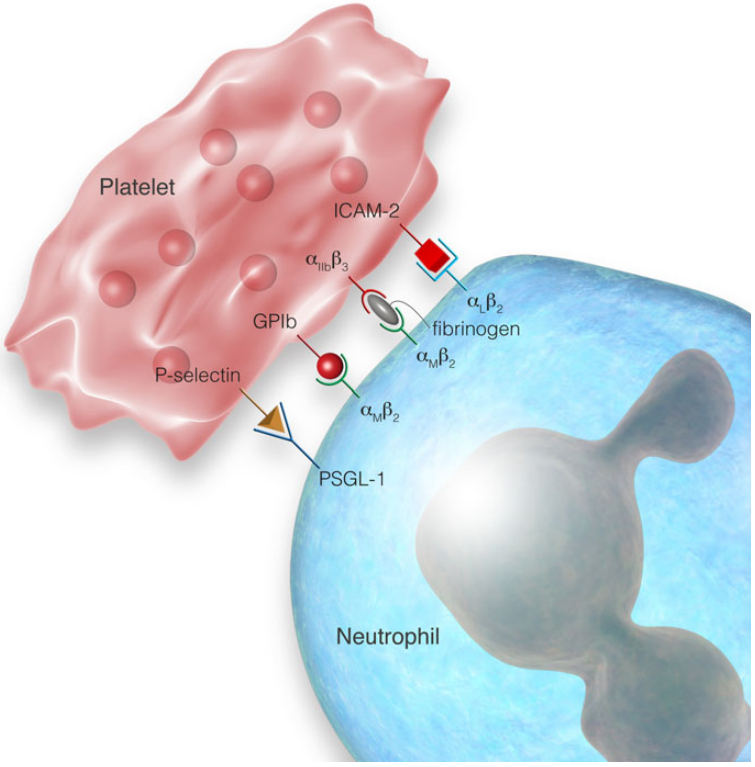

Figure 1 Adhesion molecules mediating the direct interaction between neutrophils and platelets.

2.2.2 Selectins

Selectins are a family of cell-adhesion molecules that are expressed on several cells including platelets, neutrophils, and vascular endothelial cells.[39] Neutrophils express L-selectin, platelets P-selectin, and vascular endothelial cells express both E- and P-selectin under inflammatory conditions. Resting platelets have an intracellular store of P-selectin within their α-granules. Upon platelet activation, P-selectin is incorporated into the plasma membrane, which may subsequently bind to its ligand PSGL-1 on neutrophils and monocytes with a high affinity (*Figure 1*).[5,40–44] The interaction of platelet P-selectin with PSGL-1 on neutrophils is thought to mediate the very first interaction between both cell types.[45,46] In addition, PSGL-1 engagement also initiates an intracellular signalling cascade leading to the activation of neutrophils and integrins expressed on neutrophils. This process involves the activation of the neutrophil β_2-integrins $\alpha_L\beta_2$ (LFA-1) and $\alpha_M\beta_2$ (Mac-1).[47–50] Although it has been suggested that Mac-1 is activated during this cell–cell interaction, it is still unknown whether Mac-1 on neutrophils is activated by PSGL-1, chemokine-receptors, or integrin-mediated outside-in signalling. Src family kinases (SFKs), Syk, Bruton's tyrosine kinase (BTK), and mitogen-activated protein kinases are signalling molecules that are activated in neutrophils following PSGL-1 engagement.[46,51,52] Activated Mac-1 binds to GPIb or GPIIb/IIIa on platelets via fibrinogen, stabilizing the cell–cell interaction (*Figure 1*).[53,54] In addition, activated LFA-1 may also bind to Intercellular Adhesion Molecule (ICAM)-2 expressed on platelets, which may further stabilize the cell–cell complex and promote prolonged attachment (*Figure 1*).[55–57] GPIIb/IIIa does not bind ligands under resting conditions and requires activation so that the interaction of Mac-1 with GPIIb/IIIa requires platelet activation. Furthermore, neutrophil Mac-1 may also bind to GPIbα on platelets and the importance of this interaction for leucocyte recruitment has been demonstrated in a mouse femoral artery injury model,[54,58] and for platelet-mediated neutrophil adherence to immobilized fibrinogen.[59]

Endothelial P-selectin has been demonstrated to mediate platelet rolling in both arterioles and venules in acute inflammatory processes.[60,61] However, uncertainties prevail as to the nature of the ligand for endothelial selectins on platelets, with the GPIb/IX/V complex and GPIbα being promising candidates.[62] In addition, platelets also express low amounts of PSGL-1 which may mediate platelet–endothelial interactions *in vivo*.[63] P-Selectin has been shown to be of great importance for the innate immune response under various inflammatory conditions.[7]

2.2.3 Other molecules

Besides the expression of selectins, integrins, and glycoproteins, platelets also express further surface molecules which may be involved in the interaction with leucocytes and endothelial cells. Platelets carry various members of the Immunoglobulin superfamily cellular adhesion molecules, such as ICAM-2, the junctional adhesion molecules (JAM-A, JAM-C), and the platelet endothelial cell-adhesion molecule-1 (PECAM-1).[32] These molecules interact with other cells through a mix of haemophilic (PECAM-1, JAM-A) and heterophilic interactions (ICAM-2, JAM-A, JAM-C), whereby these adhesion molecules serve as ligands for integrins.

2.3 Platelet receptors

Platelets express a variety of receptors on their cell membrane. These include various pattern recognition receptors such as members of Toll-like receptors (TLR1-9), complement receptors, and receptors for the detection of immunoglobulins (FcR).[5,64,65] Several studies have

proved the functional integration of these molecules in platelet activation. These receptors enable platelets to detect exogenous pathogens as well as to collect endogenous activating signals and subsequently integrate these stimuli in an adequate response.[65] TLRs are recognition receptors for motifs of exogenous pathogens, so called pathogen-associated molecular patterns. These motifs are evolutionarily highly conserved and the recognition by TLRs aids in the generation of an appropriate immune response.[66] Platelets express functional TLR4.[65] TLR4 on platelets recognizes lipopolysaccharide (LPS), a component of the cell membrane of gram-negative bacteria.[67] Unlike other platelet receptors, TLR4 engagement does not cause direct platelet activation and aggregation.[65,68] However, LPS-induced inflammation in mice leads to significant accumulation of platelets in the lung and in the liver.[67] The reasons for this platelet accumulation are not fully understood. It was also proposed, that platelet TLR4 is needed for the production of TNF-α during systemic infections and that engagement of platelet TLR4 increases the phagocytosis by mononuclear cells.[69] Clark *et al.*[9] showed that ligand binding to TLR4 during severe bacteraemia induced the binding of platelets to neutrophils, which caused the release of neutrophil extracellular traps (NETs) and the entrapment of circulating bacteria from the blood. However, the exact molecular interactions between neutrophils and platelets following platelet TLR4 activation remain unidentified.

2.3.1 Prostaglandin receptors

Prostaglandins are lipid-derived products that regulate a large number of physiological functions of different systems in the body (e.g. central nervous, cardiovascular, and immune system). Platelets express different receptors for prostaglandins, including thromboxane receptors, prostacyclin (PGI_2), PGD_2, and PGE_2 receptors, which have important modulatory functions. Platelets express thromboxane A_2/prostaglandin H_2 (TxA_2/PGH_2) receptors with a molecular weight of 57 kDa. Engagement of these receptors leads to phospholipase A_2 and phospholipase C activation, which subsequently amplifies platelet activation by an autocrine mechanism. Prostacyclin (PGI_2) receptors are the major inhibitory prostaglandin receptors on platelets and keep the platelets in the resting state.[70] PGE_2 receptors initiate platelet activation at low concentrations of ADP and collagen while higher concentrations of these agonists inhibit PGE_2-mediated platelet activation. TxA_2 has been shown to be the major prostaglandin involved in the regulation of inflammatory processes. TxA_2 liberation contributes to the activation of endothelial cells and amplification of inflammation.[7,71] While the role of thromboxane signalling for the pathogenesis of inflammation is well known, uncertainties exist regarding the exact source of this prostaglandin. Platelets are able to produce TxA_2 by cyclooxygenases, but it has been demonstrated that platelets alone are lacking sufficient substrates. These substrates originate from membrane phospholipids and are initially provided by the phospholipase A_2, which produces arachidonic acid. Previous research demonstrated that thromboxane generation from activated platelets is substantially higher when platelets are co-incubated with neutrophils and that the prostaglandin synthesis in activated platelets relies, at least in parts, on the availability of arachidonic acid metabolites through transcellular metabolism.[72] Interestingly, platelet P-selectin seems to play a role in the process of transcellular metabolism, as blocking P-selectin reduced thromboxane synthesis in a platelet–neutrophil co-culture.[73] However, how exactly this process is regulated remains unknown and warrants further research. The enzyme cyclooxygenase is required for thromboxane synthesis and blocking this enzyme reduced platelet TxA_2 generation, inflammation, platelet P-selectin expression, and the number of circulating platelet–leucocyte aggregates.[74]

2.3.2 Cytokines, chemokines, and chemokine receptors

Many molecules stored in platelet granules, such as platelet-derived growth factor, ADP, and thromboxane A_2, are required for haemostatic functions. For example, platelet-derived growth factor has been shown to be important in initiating and regulating wound healing at sites of inflammation.[75] However, platelet granules also contain various pro-inflammatory and anti-inflammatory cytokines and chemokines that have no clear role in haemostasis. For example, platelets contain the largest amount of transforming growth factor-β (TGF-β) in the body.[76] Although the role of this potent immunosuppressive factor in platelet-mediated haemostasis is unclear, circulating platelets appear to be important for regulating blood levels of TGF-β. Patients with immune thrombocytopenia have low levels of circulating TGF-β, but following therapy to restore normal platelet counts, their TGF-β levels recover.[77,78] The functions of the other cytokines and chemokines stored by platelets are not yet fully understood, but this is an active area of research. The chemokines β-thromboglobulins (CXCL7), platelet factor 4 (CXCL4) and RANTES (CCL5) are stored in platelets and are released immediately upon activation. These soluble factors may orchestrate neutrophil function during host defence.[79] Neutrophil-activating peptide-2 (NAP-2, CXCL7) is the truncated product of connective tissue-activating peptide III (CTAP-III) and platelet basic protein (PBP) that acts via the high affinity CXCR2 receptor. A recent study demonstrated that platelet-derived CXCL7 binding to neutrophil CXCR2 modulates the interaction of platelets and neutrophils. Blockade of CXCR2 or CXCL7-deficiency significantly decreased this interaction *in vitro* and *in vivo*.[80] CXCL7 induces more neutrophil chemotaxis compared with CXCL4 and PBP.[81] The interaction of these platelet-specific chemokines with neutrophils is tightly regulated. The CXCL7-precursor CTAP-III is able to down-regulate surface-expressed CXCL7 binding sites, predominantly CXCR2, on neutrophils and thus desensitizing neutrophils for CXCL7-stimulation. In addition, the alternative CXCL7 precursor PBP has been reported to be more potent than CTAP-III in desensitizing neutrophil degranulation and inducing neutrophil chemotaxis.[82] These studies provide evidence of the existence of negative-feedback regulation of platelet chemokine-induced neutrophil activation. However, such mechanisms may be restricted to particular steps of the recruitment process, since CXCL7 (and IL-8, CXCL8)-induced trans-endothelial migration of neutrophils is not modulated by CXCL4.[83] Interestingly, in a mouse model of acute lung injury (ALI), neutrophil recruitment and activation was dependent on the platelet-derived chemokines CCL5 (RANTES) and CXCL4 (PF-4).[8,84] These chemokines interact in a heterophilic manner to promote neutrophil arrest on inflamed endothelium.[84] CCL5 and CXCL4 are co-deposited by platelets at site of inflamed endothelium[85–87] and induce neutrophil recruitment. The involvement of platelets in neutrophil activation and recruitment is further complicated, because platelets induce the secretion of CXCL8 and CXCL1 from endothelial cells via an IL-1 dependent process.[11,88–90] Subsequent neutrophil adhesion and transmigration in response to CXCL8 has been shown to be dependent on CD11b/CD18 expression on neutrophils.[11,88–90]

Apart from liberating chemokines, platelets also express chemokine receptors on their cell surfaces,[91] including the chemokine receptors CCR1, CCR3, CCR4, CXCR4, and CX3CR1.[92,93] Platelets may be activated by ligand binding to these receptors, such as CCL17 (TARC), CCL22 (MDC), and CXCL12 (SDF-1).[94] Activated endothelial cells

may liberate CXCL13, and activated monocytes and macrophages may be the source of CCL17 and CCL22. Chemokine receptor activation leads to platelet integrin activation and incorporation of P-selectin in the platelet plasma membrane, which enables the interaction of platelets with leucocytes. Furthermore, chemokine stimulation may lead to platelet degranulation. Another implication of chemokine signalling is the induction of platelet migration. While platelets were once considered to remain stationary after adhesion, current research has identified the ability of adherent platelets to migrate along chemokine gradients *in vitro*, with CXCL12 binding to platelet CXCR4 being the driving chemokine responsible for this phenomenon.[95,96] However, further research on this topic is required to investigate the physiological relevance of this process in inflammatory disorders *in vivo* and the implications on the interaction between platelets and leucocytes.

2.3.3 Adenosine diphosphate receptors

ADP is stored in platelets in dense granules and may be released following stimulation. Compared with other platelet-agonist, ADP only weakly activates platelets. Two types of ADP receptors on platelets are known so far. The first family is the P2Y receptor family. The receptors of this family are GTP-coupled, and members of this family include the receptors $P2Y_1$ and $P2Y_{12}$. The second ADP receptor on platelets is $P2X_1$, which is an ion channel. ADP binding to $P2X_1$ leads to calcium influx into platelets, which in turns causes shape change of platelets. Apart from being a platelet agonist by its own, co-stimulation with ADP also amplifies the platelet activation by other platelet agonist. Furthermore, platelet activation by ADP also leads to production of thromboxane A_2 (TxA_2), $\alpha_{IIb}\beta_3$ activation, and platelet aggregation.[97] The participation of adenosine nucleotides in inflammation has been well studied,[98,99] and signalling through ADP receptors has been shown to play an important role in the pathogenesis of various inflammatory conditions such as sepsis.[100,101]

2.4 Consequences of the interaction of platelets with leucocytes

It has been demonstrated that the rheological displacement of red blood cells in the centre direction of the vessel promotes the formation of platelet–neutrophil aggregates *in vivo*.[102,103] Due to the blood viscous properties during flow, the smaller platelets are pushed towards the vessel walls, whereas the larger red blood cells stay centred. Thus, leucocytes at the site of the vessel wall are more likely to encounter platelets, enhancing the possibility of collisions between platelets and neutrophils. Eventually, these collisions may lead to the formation of temporary platelet–neutrophil complexes, but in the absence of an inflammatory stimulus these complexes remain temporary.[103] Furthermore, these temporary complexes do not show an increased tendency to adhere to the endothelial cell layer and the presence of a baseline amount of temporary complexes under physiological conditions has been demonstrated in healthy individuals.[104,105] The main interaction between platelets and neutrophils appears to be mediated by the binding of platelet P-selectin to PSGL-1 on leucocytes (*Figure 1*) and PSGL-1 engagement causes migration and an inflammatory response by neutrophils.[106] Activated platelets adhering to endothelial cells may increase neutrophil capturing by increasing the amount of presented P-selectin.[107]

Neutrophil recruitment has been shown to be increased by the formation of platelet–leucocyte aggregates.[7,8] In a variety of inflammatory models, this interaction has even proved to be an essential prerequisite for neutrophil recruitment.[7] However, the physiological role of this interaction does not appear to be solely limited to mechanical functions of secondary neutrophil capturing. Beyond the direct interaction of platelets with neutrophils, platelets may also be involved in maintaining the integrity of the vascular endothelium, and thus indirectly modulate neutrophil recruitment.[108] Furthermore, the interaction of platelets with neutrophils has been demonstrated to enhance cell-autonomous functions in neutrophils involved in the defence and containment of invading exogenous pathogens.[8]

The generation of reactive oxygen species (ROS) by neutrophils contributes to pathogen killing. Neutrophil ROS generation may be facilitated by a number of stimuli, including outside-in signalling via integrins and chemokine stimulation, but the formation of platelet–neutrophil aggregates also seems to contribute to ROS generation as the ROS generation capacity of neutrophils significantly increased when neutrophils form hetero-aggregates together with platelets.[109,110] The binding of platelet P-selectin to PSGL-1 on neutrophils contributes to the amplification of ROS generation by neutrophils, as inhibition of this interaction significantly lowered ROS production *in vitro*.[111] Evidence for the P-selectin/PSGL-1-dependent amplification of the ROS generation by neutrophils was also demonstrated *in vivo*.[112] However, besides the direct inhibition of the P-selectin/PSGL-1 interaction, the inhibition of the ADP receptor $P2Y_{12}$ by clopidogrel also causes decreased neutrophil ROS generation.[113] Platelet activation by $P2Y_{12}$ leads to increased P-selectin surface expression on the platelet plasma membrane, but as platelet activation via $P2Y_{12}$ also activates various other platelet-adhesion molecules, a contribution of neutrophil binding ligands other than P-selectin as well as a necessity of platelet autoactivation by an ADP-feedback loop might be possible. Besides ROS generation, the phagocytic capacity of neutrophils as the second instrument for pathogen elimination may also be modulated by platelet–neutrophil interactions. A substantial body of published research suggests that platelet-derived mediators rather than the actual physical interaction may be responsible for this effect, and they postulated that platelet-derived purine nucleotides and prostaglandins might be involved.[114–117] However, this concept was questioned by recent results that demonstrate that efficient neutrophil phagocytosis of bacteria *in vivo* during periodontitis also requires platelet (but not neutrophil) TLR2 and the formation of platelet–neutrophil aggregates.[118] Further research in this field is needed to understand the contribution of platelet–neutrophil interactions on neutrophil phagocytic capacity *in vivo*.

The discovery of NETs as the third anti-bacterial measure by which neutrophils actively combat pathogens was made in 2004 by Brinkmann *et al.*[13] NETs are webs of extracellular DNA and histones. Moreover, proteolytic and bactericidal proteins from neutrophil granules are integrated in these structures. NETs are produced by neutrophils in a process called 'NETosis'. While implying a form of cell death, neutrophils undergoing NETosis may still be viable after they have actively decondensed their nuclear chromatin to form NETs and may still retain cell motility for intravascular crawling and migration within tissues.[119] Circulating bacteria are efficiently captured by NETs, thus NETs may be seen as effective tools for the removal of circulating pathogens during sepsis.[10] However, the formation of NETs has also been demonstrated during aseptical inflammatory disorders, and current research has also indicated a direct effect of NETs on neutrophil recruitment.[8,9,12] The interaction of platelets and neutrophils has been shown to be involved in NET formation (*Figure 2*). Several molecular mechanisms for platelet-mediated NET formation have been identified. Platelet TLR4 has been previously identified to be involved in platelet activation in response to LPS. TLR4-stimulation of platelets induces complex formation with

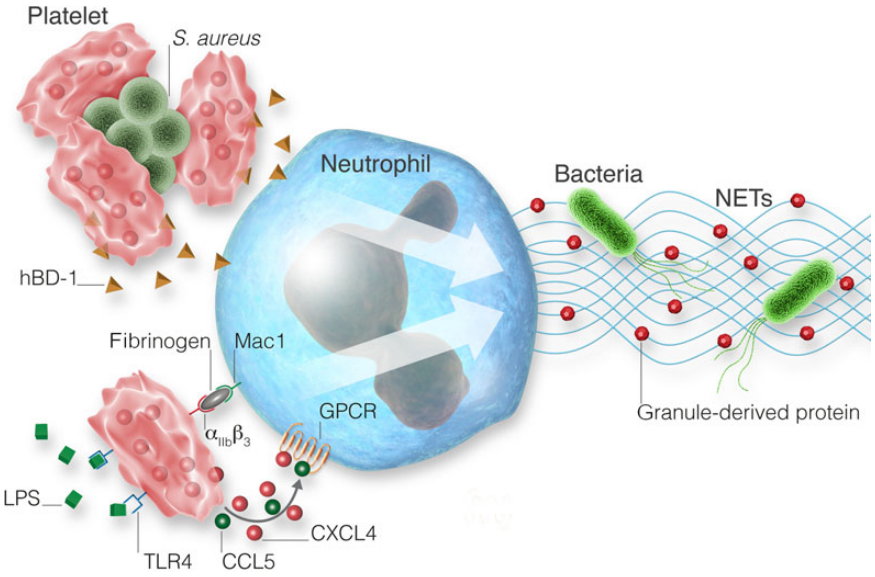

Figure 2 Mechanisms of platelet-mediated NET formation.

neutrophils leading to NET formation (*Figure 2*).[9] Another important platelet-derived mediator of NET formation is human β-defensin 1 (hBD-1). This peptide is released by platelets following exposure to *Staphylococcus aureus* α-toxin and may induce NET formation without the necessity for a direct, physical interaction of platelets with neutrophils (*Figure 2*).[120] Current research could also demonstrate that the direct interaction of platelets and neutrophils is essential for platelet-mediated NET formation.[8,12] The binding of platelet integrin $\alpha_{IIb}\beta_3$ to neutrophil CD11b via fibrinogen causing outside-in signalling into neutrophils together with the synchronized chemokine-stimulation of neutrophils by the CXCL4/CCL5 heteromer could be identified as a necessity of NET formation during sterile inflammation.[8] Beyond acute inflammatory processes, NET formation is also involved in coagulatory disorders such as deep-vein thrombosis (DVT). The formation of intravascular thrombi during DVT in mice does not only include plasmatic coagulation and platelet activation, but also includes leucocytes. Here, neutrophils form GPIbα-dependent aggregates with platelet, which leads to subsequent NET formation. Although the precise role of NETs in DVT has not been identified yet it could be shown that preventing the interaction of neutrophils and platelets consequently abolished NET formation, which prevented the formation of stable, occlusive thrombi.[121] In accordance with these findings, intravascular NETs were also found to be pro-thrombotic.[122]

2.4.1 Platelet microparticles

Microparticles are small vesicles (diameter about 0.1–1 μm) that may originate from almost all eukaryotic cells following activation.[123] They were originally considered to be cell remnants without function, but recent research discovered that microparticles fulfil a variety of biological functions.[124] Under physiological conditions, most microparticles in the circulation are derived from platelets.[125] Interestingly, proteome studies have revealed that although platelet-derived microparticles share many proteins with platelets, a substantial amount of proteins were exclusively expressed in microparticles.[126] These results support the conclusion that the generation of platelet-derived microparticles is not just a passive process, but requires very distinct and active molecular pathways in platelets that still have to be identified. Due to the presence of cell-surface receptors on platelet-derived microparticles they are also able to interact with leucocytes.[127,128] In the human system, platelet-derived microparticles present numerous cell-surface molecules which are also expressed by platelets. These molecules include P-selectin and the platelet integrin $\alpha_{IIb}\beta_3$.[129] Platelet-derived microparticles are able to bind to different cell types. Additionally, microparticles may also activate endothelial cells, leucocytes, and other platelets. Thus, platelet-derived microparticles may serve as inflammatory mediators and may also be able to transduce inflammatory signals by release of soluble mediators, e.g. CCL5.[130]

2.5 Platelet-dependent acute inflammatory diseases

2.5.1 Acute lung injury

Acute lung injury is a life-threatening disease that develops due to several causes, including direct injury to the lung by bacterial infection or aspiration of gastric content or due to indirect causes such as sepsis.[131] Hallmarks of acute lung injury are the recruitment of immune cells, e.g. neutrophils, into the lung and the increase of vascular permeability and subsequent oedema formation.[132,133] Acute lung injury has been shown to be platelet-dependent in many disease models, including LPS-induced lung injury,[84,134] ventilator-induced lung injury (VILI),[8] acid-induced lung injury,[7] and transfusion-related acute lung injury (TRALI).[6,12,135] Platelet–neutrophil aggregates can be found in the lung microvasculature during inflammation and acute lung injury (*Figure 3*). Following the intratracheal instillation of acid, increased amounts of circulating platelets could be detected as early as 30 min after acid instillation, and platelet P-selectin has been shown to be required for this process.[7] Platelet depletion or P-selectin blockade decreased neutrophil recruitment and edema formation. Interestingly, the interaction of platelets and neutrophils was also necessary for thromboxane A_2 generation, which induced the up-regulation of endothelial ICAM-1. A recent study demonstrated that the pathogenesis of

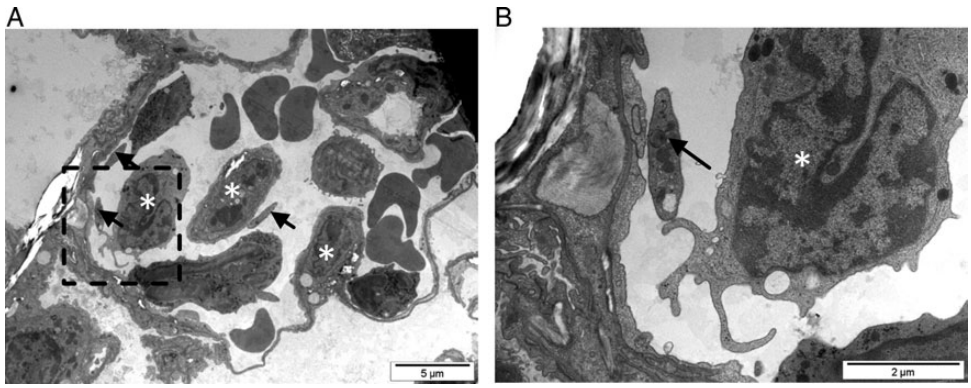

Figure 3 Platelet–neutrophil aggregates in the lung microvasculature during acute lung injury. (*A*) Transmission electron microscopy image of a lung capillary 4 h after intratracheal instillation of *Escherichia coli* bacteria. Neutrophils are marked with asterisks and platelets are marked by black arrows. (*B*) Higher magnification of dashed insert.

VILI also involves the formation of platelet–neutrophil complexes. This cell–cell interaction triggered the formation of NETs which was necessary for neutrophil recruitment.[8] The importance of platelet–neutrophil interactions for leucocyte recruitment during acute lung injury has also been shown in another model of ALI.[84]

The significance of TRALI in transfusion medicine has been recognized recently, as it has been ranked as one of the leading causes of transfusion-related complications.[135] Several studies investigating the role of platelets in lung injury have suggested that these cells may be involved in the pathogenesis of TRALI.[109] Ligation of TLRs on platelets leads to the release of CD154,[136] whereas soluble CD154 may promote activation of pulmonary neutrophils through CD40 engagement, thereby contributing to TRALI.[137] In all aforementioned models of acute lung injury, the inhibition of platelet–neutrophil complex formation was associated with an improved outcome. This can be explained by the fact that all models are aseptical and do not involve the presence of exogenous pathogens. However, in studies using acute lung injury models induced by bacteria, the depletion of platelets were associated with a worse outcome due to unregulated bacterial growth and dissemination.[138]

While it is now commonly accepted that the interaction of platelets and neutrophils is a prerequisite for neutrophil recruitment in many entities of acute lung injury, uncertainties whether this interaction is restricted to the intravascular compartment remain. In a recent study, Ortiz-Munoz *et al.*[139] presented first evidence that suggests that platelet–neutrophil complexes are dynamically formed in the blood during acute lung injury and that at least some of this complexes can also be found in the intraalveolar space. The exact understanding of the dynamics, location, and pathophysiological role of platelet–neutrophil complexes during acute lung injury remains the subject of active research.

2.5.2 Acute hepatic injury

The liver is the first organ that is perfused with blood coming from the intestines. Because the intestinal tract represents a major entrance port for pathogens, the liver is continuously exposed to blood-borne pathogens in the microcirculation, and current research has recognized that the liver as a major immunological barrier protecting the host from bacteraemia.[140] Platelets contribute to the immunological processes in the liver and it has been shown that platelets act as physiological immune sentinels and scan the liver sinusoids for pathogens presented by Kupffer cells, and subsequently boostering neutrophil recruitment and the innate immune response.[14] Platelets have also been demonstrated to facilitate the recruitment of T cells into the liver during hepatic injury following viral hepatitis.[141] Vasoactive, platelet-derived serotonin has been identified as a mediator of reduced sinusoidal perfusion and T cell recruitment in this model.[142] Interestingly, hepatic viral infections also induce the recruitment of platelets and neutrophils into the liver, inducing the formation of NETs which seem to play a role in controlling viral infection.[143,144]

2.5.3 Sepsis

Sepsis in critically ill patients is commonly associated with thrombocytopenia and increased mortality.[145] During the course of disease, leucocytes and platelets are activated which may lead to disseminated intravascular coagulation and multi-organ failure as a consequence of diminished tissue perfusion and the initiation of an pro-inflammatory cascade involving different cytokine pathways.[146] In addition, a high P-selectin expression on the cell surface of activated platelets in septic patients is associated with a higher abundance of platelet microparticles.

3. Conclusion

Platelets are traditionally known for their role in haemostasis, but their participation in the innate immune function may be of similar importance. So far, a growing body of evidence contributed to the detailed understanding of the molecular mechanisms governing the interaction of platelets with leucocytes under inflammatory conditions. Future studies will have to investigate the contribution of platelets to individual inflammatory processes in different organs. This knowledge is necessary for the development of specific therapeutic strategies for the control of inflammatory diseases involving platelets.

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