Immune functions of platelets

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Summary
This review collects evidence about immune and inflammatory functions of platelets from a clinician’s point of view. A focus on clinically relevant immune functions aims at stimulating further research, because the complexity of platelet immunity is incompletely understood and not yet translated into patient care. Platelets promote chronic inflammatory reactions (e.g. in atherosclerosis), modulate acute inflammatory disorders such as sepsis and other infections (participating in the host defense against pathogens), and contribute to exacerbations of autoimmune conditions (like asthma or arthritis). It would hence be obsolete to restrict a description of platelet functions to thrombosis and haemostasis – platelets clearly are the most abundant cells with immune functions in the circulation.

Keywords
Platelet immunology, sepsis, leukocyte function / activation

Introduction
The primary mission of platelets is haemostatic, because they mediate primary haemostasis and atherothrombotic events (reviewed in [1]). Platelets also promote venous thromboembolism (2, 3). But platelets not only build thrombi, they are also highly active in the host defense against pathogens and have hence been recognized as cells with immune functions. They are involved in sepsis and other acute inflammatory responses to infection as well as in chronic inflammatory diseases like atherosclerosis or arthritis (reviewed in [4–8]). Platelets circulate in large numbers through the vasculature (several hundred thousand per microliter), patrolling the endothelium and are able to rapidly release an array of immunomodulatory cytokines, chemokines, and other mediators (9, reviewed in [10]). Platelets express several immune receptors on the cell surface, such as Toll-like receptors, immunoglobulin receptors or the co-stimulatory proteins CD40 and CD40L. Another feature that platelets have in common with other immune cells is the presence of granules in the cytoplasm that can be exocytosed following specific stimulation. This allows the targeted release of mediators and the instantaneous upregulation of surface receptors at sites of inflammation. Platelets are able to modulate leukocyte functions such as phagocytosis or extravasation directly or in cooperation with endothelial cells of inflamed vessels. Surprisingly, increasing evidence shows that platelets are even able to capture and neutralise pathogens directly. Here we summarise observations of immune and inflammatory platelet components and functions.

Clinical and experimental evidence of immune functions of platelets
Every clinician has experienced marked changes in platelet count of patients with inflammatory diseases and many may have speculated about the role that platelets play in immunity. Whether long-term antiplatelet therapy, especially with the newer, more potent agents, influences inflammatory or immune responses has not been examined systematically. However, several studies have deciphered relevant contributions of platelets to the pathophysiology of inflammatory or immune disorders in animals or humans.

Infections
Thrombocytopenia is a common feature of severe bacterial or viral infection and a marker of poor outcome (reviewed in [11]). This suggests that platelets actively participate in the struggle against pathogens. Interestingly, recovery is often associated with reactive thrombocytosis (12). Platelet consumption in microthrombotic responses to infection is a possible complication, such as in disseminated intravascular coagulation (DIC), but does not explain these frequent findings in milder clinical courses. Thrombocytopenia and reactive thrombocytosis are hence not only collateral damage, but most likely constitute an active part of the host defense against infection. Curiously, direct antimicrobial activity of platelets has been discovered in animal models. In murine malaria, platelets were shown to eradicate intra-erythrocytic parasites and have hence an impact on survival, an effect that could be reproduced in ex vivo models with human blood cells (13). When platelet binding to hepatic Kupffer cells via glycoprotein (GP)Ibα was imaged...
in intravital microscopy of mice, challenging these mice with bacteria resulted in firm platelet immobilisation via GPIIb and encapsulating of bacteria (14).

Still, direct clinical evidence from human studies is required before preclinical data can be translated into therapeutic interventions. It is possible that cessation of antiplatelet therapy improves infectious disease outcome, but this assumption is far from evidence-based and would likely be associated with thrombotic complications. In fact, to our knowledge, antiplatelet therapy has not been linked to an increase in infectious disease prevalence or morbidity. Moreover, there are no epidemiologic data showing that platelet suppression affects infections. In addition, patients with GPIb deficiency in Bernard-Soulier syndrome are not known to suffer from immune defects. Of note, however, a recent tragic case report suggested an association between GPIIb/IIIa deficiency in Glanzmann thrombasthenia and human immunodeficiency virus (HIV) susceptibility (15).

The key to success in translational research is therefore further mechanistic research. Targeted – positive – platelet modulation (e.g. functional enhancement of GPIIb or specific modulation of intracellular signaling [16]) could theoretically complement anti-infectious therapy, but this has not been addressed systematically. More applicable however appears the prospect of platelet function suppression in the context of inflammatory and (auto-) immune disorders to attenuate pathological systemic inflammatory responses (e.g. in sepsis, asthma, or atherosclerosis).

**Sepsis**

In human sepsis, the number of circulating platelet-neutrophil complexes (PNCs) and platelet-monocyte complexes (PMCs) increases dramatically (17), correlating with the severity of multi-organ failure (18). A differential release of growth factors from platelets was observed in septic patients (19): The haemostatic functions were attenuated in relation to the severity of sepsis but the release of vascular endothelial growth factor (VEGF) was enhanced. Moreover, the transcriptome is altered in platelets from septic patients, facilitating differential release of proteins such as tissue factor (20). Microthrombotic complications are provoked by disseminated platelet activation and platelet-leukocyte interactions, mechanisms that may both be targeted in future therapeutic trials (17, 21, 22).

**Autoimmune disease**

Immune complex formation and complement activation by platelets were found in patients with immune thrombocytopenia and systemic lupus erythematosus [reviewed in (23)]. Platelets release serotonin into acutely inflamed joints in murine rheumatoid arthritis, increasing synovial permeability (24). Both mechanisms may represent attractive targets for therapeutic intervention.

**Asthma**

Platelet activation and granule secretion enhance bronchoconstriction and -obstruction during asthmatic attacks in mice and humans (25–29). ATP and serotonin are released from dense granules sustaining these attacks (30, 31). Bronchoalveolar lavage fluid after segmental allergen challenge of asthmatic patients contains high levels of platelet-derived serotonin, which enhances leukocyte infiltration, Th2-promising capacity of dendritic cells (DCs), and ultimately all cardinal features of allergic airway inflammation (31). Antiserotonergic and antipurinergic intervention consequently represent promising strategies in the search for future therapies.

**Metabolic syndrome**

Data from the Framingham heart study suggest that inflammatory platelet activation correlates with obesity and cardiovascular risk (32, 33). Inflammatory gene transcripts derived from isolated platelets such as tumour necrosis factor (TNF), toll-like receptor (TLR)2, and TLR4 were associated with increased body mass index (BMI) (33). It is hence conceivable, that platelets promote the inflammatory phenotype of metabolic syndrome, which in turn could be targeted in future applications (34).

**Atherosclerosis**

Platelets not only drive the atherothrombotic occlusion of a coronary artery in acute myocardial infarction, but they also mediate the chronic progression of vessel wall inflammation in atherosclerosis (37) [reviewed in (35, 36)]. The various aspects ranging from cytokine release to monocyte recruitment have been examined in several in-depth animal studies and complemented by human *ex vivo* data [reviewed in (7)]. Specific antiplatelet intervention to stop the atherogenetic progression is as desirable as it is unavailable at present. Several research projects, including clinical studies currently address this topic.

**Ischaemia / reperfusion injury**

Ischaemia / reperfusion (IR) injury contributes to the final infarct size in myocardial infarction and is still poorly controlled. PNC infiltration correlates with myocardial reperfusion damage (38) and PMCs form rapidly after myocardial infarction in animal studies [reviewed in (21)]. It has been reasoned that the adenosine diphosphate (ADP) receptor P2Y12 inhibitor ticagrelor may possess pleiotropic effects and could limit IR injury (39, 40). IR injury of the liver is also mediated by platelet-neutrophil interactions in mice (41), while liver regeneration depends on platelet-derived serotonin (42). Whether specific antiplatelet intervention could limit IR injury in patients with myocardial infarction remains to be shown. This is of particular interest, because although reperfusion is often rapidly ensured by percutaneous coronary intervention, the subsequent inflammatory sequelae still dictate the final extent of the myocardial scar and we lack tools to limit them.
Soluble factor secretion

Platelets have a number of immune and inflammatory features ranging from secretable immunomodulatory factors to stably or variably expressed surface receptors (summarised in Table 1). Rapid secretion of a wide array of inflammatory mediators following exocytosis of α granules, dense granules, and lysosomes upon activation is a unique feature of platelets. This enables an almost instantaneous response to stimuli in the affected vasculature. Of note, platelets not only contain preformed secretable factors, but are also capable of newly synthesising mediators such as interleukin (IL)-1β following signal-dependent splicing of pre-mRNA (43).

α-granule factors

α-granules are the most abundant granules in platelets and contain a variety of inflammatory mediators, including a number of adhesion proteins (45). Platelet aggregation and (micro-)thrombus formation are promoted by fibrinogen, von Willebrand factor (VWF), fibronectin, vitronectin and serve as an immobilising matrix for pathogen capture [reviewed in (21, 44, 45)]. Theoretically, this limits pathogen growth and multiplication in the vasculature and facilitates exposure of these captured pathogens to neutralising leukocytes. Although this pathophysiologic sequence has not been deciphered directly in mechanistic studies, observations in septic patients (and mice) suggest that platelet aggregation is not only a complication but rather a feature of primary host defense (17). Platelet factor 4 (PF4, CXCL4) and the β-thromboglobulin neutrophil-activating protein 2 (NAP2, CXCL7) regulate neutrophil and monocyte functions and promote their recruitment (89). PF4 furthermore suppresses neutrophil apoptosis, which was demonstrated in a platelet depletion study of murine limb ischaemia (140). Several α-granule-derived chemokines have been studied extensively, especially in atherogenesis, and are considered important messengers of immune functions [reviewed in (141)]. Chemokine functions include chemotaxis and modulation of different leukocyte functions. Platelets are able to take up immunoglobulins from plasma to store them in α-granules and to release this cargo on-site following inflammatory stimulation (44, 142) [reviewed in (44)]. This interesting observation may represent a contribution of platelets to humoral immunity.

Dense granule factors

Dense granules store serotonin, calcium, magnesium, ATP, and ADP (whether they also contain histamine is controversial [93, 143]) and secrete these factors upon activation [reviewed in (143, 144)]. At the site of acute inflammation platelets release serotonin at micromolar concentrations, boosting the recruitment of neutrophils into the inflamed tissue during murine pneumonia, peritonitis, and skin wounds (91). In mice, this translates into improved sepsis outcome when platelet serotonin stores were depleted. The observation that antidepressants inhibiting the uptake of serotonin modulate the release of several cytokines, suggests that platelet serotonin may also be important in human inflammation [reviewed in (145)]. In fact numerous immunomodulatory functions of peripheral serotonin (which is mostly platelet-derived) have been characterised, including differential effects on chemokine/cytokine secretion by immune cells. Figure 1 summarises immune effects of serotonin as one example of the complexity of platelet immunomodulation (24, 31, 91, 146–165).

Lysosomes

Lysosomes release glycosidases, proteases, and bactericidal enzymes such as β-glucuronidase, elastase, and collagenase [reviewed in (46)]. The lysosome releasate facilitates pathogen clearance and breakdown of extracellular matrix but current studies are rare and clinical implications have not been addressed systematically.

Defensins

Other secretable factors are not associated with any of the known granules. They are either derived from cytoplasmic stores, are newly synthesised proteins, or are components of a yet unknown type of granule. β-defensin is an example of granule-independent mediators with anti-bacterial activity (107) and belongs to the group of antimicrobial peptides. Human platelets express β-defensins 1, 2, and 3 (107, 166, 167). Platelets release β-defensin 1 from cytoplasm in response to Staphylococcus aureus α-toxin to induce neutrophil extracellular trap (NET) formation and limit bacterial growth (107).

Immune receptors

Different immune receptors operate on the platelet surface (and in some cases intracellularly) [reviewed in (168)]. Toll-like receptors recognise pathogen- and danger-associated molecular patterns (PAMPs and DAMPS, respectively) [reviewed in (6)], complement receptors mediate complement activation at sites of platelet accumulation (169), and Fc receptors recognising immunoglobulins (FcR, notably the Fcγ receptor FcγRIIA, but also Fcα and Fcε receptors) provide a link to the adaptive immune system (79, 170). In a murine model of Arthus reaction, platelets facilitate the immune complex-induced recruitment of neutrophils in microvessels (171). P-selectin on activated platelets appears to participate in complement activation and platelet-associated immune complexes mediate autoimmune diseases such as immune thrombocytopenia or systemic lupus erythematosus (23, 79). C-type lectin-like receptor 2 (CLEC-2) is involved in the regulation of vascular integrity in acute inflammation (119, 172).

CD40 / CD40 ligand

With cell activation differentially regulating their surface presentation, CD40 and CD40L are not only expressed by platelets, but also by endothelial cells, smooth muscle cells, and several leukocyte

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Table 1
Table 1: Immune and inflammatory platelet components [reviewed in (4, 7, 21, 44–46)].

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<tr>
<th>Superfamily</th>
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<td>α-granules</td>
<td>P-selectin</td>
<td>cell-cell interactions (leukocytes, endothelium)</td>
<td>PSGL-1 (on neutrophils, monocytes, microparticles, Th1 cells), unknown (on endothelium)</td>
<td>(10, 45, 47)</td>
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<td>Fibrinogen</td>
<td>cell-cell interactions (platelets, leukocytes, endo-</td>
<td>GPlba, GPIIb/IIIa (platelets), integrins (platelets, leukocytes), collagen (VWF)</td>
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<td>VWF</td>
<td>pathogens, bacterial trapping</td>
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<td></td>
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<td></td>
<td>Fibronecitin</td>
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<td></td>
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<td></td>
<td>Vitronectin</td>
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<td>Thrombospondin-1</td>
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<td></td>
<td>PECAM-1</td>
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<td>Coagulation</td>
<td>Factor V</td>
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<td>Monocytes, macrophages, T cells</td>
<td>(60, 61)</td>
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<td>factors</td>
<td>Protein S</td>
<td>fibrinolysis</td>
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<td>(56)</td>
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<td></td>
<td>Factor XI</td>
<td>promotion of coagulation</td>
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<td>(57)</td>
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<td></td>
<td>Factor XIII</td>
<td>fibrin stabilization</td>
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<td>(62, 63)</td>
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<td>factors</td>
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<td>EGF</td>
<td>pro-mitogenic action</td>
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<td>(64)</td>
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<td>VEGF</td>
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<td>VEGF receptors</td>
<td>(65)</td>
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<tr>
<td>factors</td>
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<td>n/a</td>
<td>(66)</td>
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<td>Protease</td>
<td>α2-plasmin inhibitor</td>
<td>fibrinolysis</td>
<td>Plasmin, neutrophil elastase</td>
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<td>inhibitors</td>
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<td>Tissue plasminogen activator</td>
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<td>Antimicrobial</td>
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<td>(73)</td>
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<td>IgM</td>
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<td>membrane-spec-</td>
<td>GMP33</td>
<td>*</td>
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<td>(76)</td>
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<td>ific proteins</td>
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<td>Chemokines</td>
<td>CCL3 (MIP-1α)</td>
<td>leukocyte activation/recruitment</td>
<td>Monocytes/macrophages, eosinophils, basophils, NK cells, DCs</td>
<td>(77, 78)</td>
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<td></td>
<td>CCL5 (RANTES)</td>
<td>monocyte recruitment</td>
<td>Monocytes, eosinophils, basophils, NK cells, T cells, DCs, platelets</td>
<td>(78–81)</td>
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<td>CXCL1 (GROα)</td>
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<td>Neutrophils / CXCR2</td>
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<td>CXCL4 (PF4)</td>
<td>monocyte recruitment and differentiation, anti-angiogenic, T cell modulation</td>
<td>Monocytes, neutrophils, T cells, platelets</td>
<td>(83–86)</td>
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<td>CXCL5 (ENA78)</td>
<td>modulation of chemokine scavenging, neutrophil chemotaxis</td>
<td>Neutrophils</td>
<td>(87, 88)</td>
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<td></td>
<td>CXCL7 (NAP2, β-</td>
<td>(most abundant platelet chemokine, several variants)</td>
<td>Neutrophils, EPCs / CXCR1,2</td>
<td>(89, 90)</td>
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<td>thromboglobulin)</td>
<td></td>
<td>neutral recruitment and activation, EPC homing</td>
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Table 1: Continued

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<td>Serotonin (5-HT)</td>
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<td>neutrophils, monocytes, lymphocytes, NK cells, platelets / 5-HT receptors</td>
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<td>Histamine (?)</td>
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<td>DCs / P2X, P2Y receptors</td>
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<td>ADP</td>
<td>second-wave platelet activation</td>
<td>platelets / P2Y receptors</td>
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<td>Collagenase</td>
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<td>(101)</td>
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<td><strong>Glyco-hydrolases</strong></td>
<td>Heparinase</td>
<td>facilitation of cell recruitment</td>
<td>endothelial glycocylix</td>
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<td>β-N-acetylglucosaminidase</td>
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<td><strong>Granule-independent soluble mediators</strong></td>
<td>CCL7 (MCP3)</td>
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<td>acute coronary syndrome                                                         CD40</td>
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<td>adhesion</td>
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<td>α2bβ3 (GPIIb/IIIa)</td>
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<td>fibrinogen, fibronectin, vitronectin, VWF, thrombospondin</td>
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<td>GPIbα</td>
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<td>VWF, P-selectin, Mac-1</td>
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<td>ICAM-2</td>
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<td>GPVI</td>
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<td>collagen</td>
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<td>CLEC2</td>
<td>vascular integrity</td>
<td>podoplanin</td>
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<td>Toll-like receptors (TLRs)</td>
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<td>*</td>
<td>heterodimerizes with TLR2</td>
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<td>gram-positive bacteria</td>
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<td>TLR4</td>
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<td>TLR7</td>
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<td>Co-stimulatory proteins (TNF and TNFR superfamily)</td>
<td>CD40</td>
<td>atherosclerosis, IR injury</td>
<td>T cells / CD40L</td>
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<td>CD40L (CD154)</td>
<td>atherosclerosis</td>
<td>CD40 on monocytes and endothelial cells / B cells, DCs, monocytes, macrophages, platelets</td>
<td>(126–129)</td>
<td></td>
</tr>
<tr>
<td>Ig superfamily</td>
<td>TREM-1 ligand</td>
<td>sepsis</td>
<td>PMNs, DCs, macrophages, monocytes</td>
<td>(130, 131)</td>
<td></td>
</tr>
<tr>
<td>Ig receptors</td>
<td>TACE (ADAM17)</td>
<td>*</td>
<td>GPIb, TNFα</td>
<td>(132)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRIIA (CD32)</td>
<td>bactericidal action</td>
<td>IgG</td>
<td>(21, 133)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRI</td>
<td>allergy, parasite defense</td>
<td>IgE (high affinity)</td>
<td>(79)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRII (CD23)</td>
<td>allergy, parasite defense</td>
<td>IgE (low affinity)</td>
<td>(134)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRI</td>
<td>IL-1β production</td>
<td>IgA</td>
<td>(135)</td>
<td></td>
</tr>
<tr>
<td>Complement components</td>
<td>gC1qR</td>
<td>antimicrobial</td>
<td>bacterial protein A</td>
<td>(136, 137)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSb-9</td>
<td>dense granule release</td>
<td>anaphylatoxins</td>
<td>(138, 139)</td>
<td></td>
</tr>
</tbody>
</table>


subtypes (4, 110, 125–129, 173–177) [reviewed in (4, 128, 129, 173, 174)]. This inflammatory axis mediates complex cell-cell interactions. CD40/CD40L-mediated interactions have been well characterised in atherosclerosis, but are likely also involved in several other immune reactions. Platelets release CD40L and RANTES upon stimulation with IgG complexes without showing signs of general activation (80). Platelet CD40L triggers inflammatory activation of endothelium: it induces upregulation of E-selectin, ICAM-1, and VCAM-1 and provokes chemokine secretion by endothelial cells (178).

**Toll-like receptors**

Platelets express functional TLRs and respond to ligand binding and activation (179–181) [reviewed in (122)]. TLR2 and TLR4 and the predominately intracellular TLR9 (182) are the most extensively studied platelet TLRs (121, 182–184). Platelets also contain the adapter molecules MYD88 and TRIF required for specific downstream signalling (185, 186). The TLR2/1-specific agonist Pam3CSK4 has been utilised by different groups to stimulate platelets. It induces a dose-dependent response involving different intracellular signalling pathways, which may be part of defense mechanisms against gram-positive bacteria (187, 188). Stimulation of platelet TLR4 has been linked to NET formation and subsequent capture of gram-negative bacteria in the blood stream (189). Platelet TLR7 binds viral RNA triggering PNC formation in mice, improving the animals’ survival (123). Platelet TLR9 recognises viral and bacterial DNA and promotes platelet reactivity (182, 190). Finally, TLR9 mediated protection from atherosclerosis in mice by suppressing the influx of CD4+ T cells into plaques (124). Platelet TLRs are an interesting target for future therapeutic studies because pharmacological manipulation is uncomplicated.
Membrane receptor shedding
Soluble CD40 ligand
Platelets are an important source of soluble CD40 ligand (sCD40L) [reviewed in (173, 174)]. Platelet-derived sCD40L induces reactive oxygen species (ROS) production, neutrophil adhesion receptor upregulation, macrophage activation, and cytotoxic T cell and B cell stimulation [reviewed in (21)]. CD40L can also be carried by platelet microparticles, regulating antigen-specific IgG production [reviewed in (21, 173)]. Whether platelet-derived sCD40L is accessible to pharmacological intervention remains to be shown.

TACE
TNFα converting enzyme (TACE, ADAM17) is a sheddase expressed not only by neutrophils (where it regulates shedding of L-selectin and pro-TNFα), but also by platelets (191, 192). Numerous signals can activate TACE, including atherosclerotic plaque components (193, 194). Although specific immune functions of platelet surface TACE have not yet been deciphered, it is intriguing to note that oxidative stress and serotonin receptor activation induce GPIbα and GPV shedding by TACE (132, 191, 195–197).

Overview of immune and inflammatory platelet functions
The different immune and inflammatory functions of platelets have been divided into four major functional classes: leukocyte modulation, leukocyte recruitment, pathogen capture, and pathogen killing [reviewed in (44)]. Table 2 allocates mechanisms to these four classes.

Interactions with endothelium and leukocytes
An early response to acute inflammation is the activation of endothelium in affected vessels. This induces platelet and leukocyte interactions with the vessel wall resulting in rolling, adhesion, and transmigration into the inflamed organ [reviewed in (10, 208)]. During inflammatory cell recruitment, platelets and leukocytes also closely interact with each other and form platelet-leukocyte complexes, predominantly PNCs and PMCs (Figure 2) (17, 209, 210) [reviewed in (21)]. A key mediator is P-selectin, which is exposed on the platelet surface after a granule exocytosis and then binds leukocyte PSGL-1 [reviewed in (45)].
Table 2: Immune and inflammatory functions of platelets [reviewed in (4, 7, 21, 44)].

<table>
<thead>
<tr>
<th>Function</th>
<th>Mechanism</th>
<th>Effector cells, targeted pathogen</th>
<th>Pathophysiological relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Modulation of leukocyte function</strong></td>
<td>PNC formation enhances phagocytosis (157, 198)</td>
<td>neutrophils, bacteria</td>
<td>periodontitis, staphylococcal infections</td>
</tr>
<tr>
<td></td>
<td>PNC formation promotes neutrophil degranulation (189)</td>
<td>neutrophils</td>
<td>sepsis</td>
</tr>
<tr>
<td></td>
<td>platelet sCD40L release activates endothelial cells and leukocytes (173, 174)</td>
<td>endothelial cells, lymphocytes, macrophages</td>
<td>atherosclerosis</td>
</tr>
<tr>
<td></td>
<td>platelet sCD40L release advances the production of reactive oxygen species (173, 174)</td>
<td>Neutrophils</td>
<td>acute inflammation</td>
</tr>
<tr>
<td><strong>Leukocyte recruitment</strong></td>
<td>PMC formation facilitates monocyte recruitment (199–202)</td>
<td>monocytes</td>
<td>thrombosis, atherosclerosis, autoimmunity</td>
</tr>
<tr>
<td></td>
<td>platelet sCD40L induces endothelial ICAM-1, VCAM-1, E-selectin expression (178)</td>
<td>endothelial cells</td>
<td>inflammation</td>
</tr>
<tr>
<td></td>
<td>platelet serotonin induces endothelial P-selectin, E-selectin expression (91, 203)</td>
<td>endothelial cells</td>
<td>sepsis, wound healing, infections</td>
</tr>
<tr>
<td></td>
<td>platelet GPIb-IX-V binding to VWF (48)</td>
<td>subendothelium</td>
<td>inflammation, thrombosis</td>
</tr>
<tr>
<td></td>
<td>platelet GPIb/IIla binding to ICAM (10)</td>
<td>endothelial cells</td>
<td>inflammation, thrombosis</td>
</tr>
<tr>
<td></td>
<td>cytokines and chemokines activate leukocytes</td>
<td>neutrophils, monocytes, macrophages, lymphocytes</td>
<td>inflammation</td>
</tr>
<tr>
<td></td>
<td>platelet GPIV binding to collagen (48)</td>
<td>subendothelium</td>
<td>inflammation, thrombosis</td>
</tr>
<tr>
<td></td>
<td>platelet P-selectin binding to neutrophil PSGL-1 (45)</td>
<td>neutrophils</td>
<td>sepsis</td>
</tr>
<tr>
<td></td>
<td>platelet GPIb and IIb/IIla binding neutrophil Mac-1 (204, 205)</td>
<td>neutrophils</td>
<td>atherothrombosis</td>
</tr>
<tr>
<td><strong>Pathogen capture</strong></td>
<td>platelet-triggered NET release by neutrophils (189)</td>
<td>neutrophils</td>
<td>sepsis</td>
</tr>
<tr>
<td></td>
<td>platelet aggregation limiting pathogen multiplication or spreading (206)</td>
<td>bacteria</td>
<td>bacterial infections</td>
</tr>
<tr>
<td></td>
<td>internalization of certain viruses and bacteria by platelets (207)</td>
<td>viruses, bacteria</td>
<td>HIV, <em>S. aureus</em></td>
</tr>
<tr>
<td></td>
<td>complement C3-mediated binding and presentation to leukocytes (116)</td>
<td>gram-positive bacteria</td>
<td>bacterial infections</td>
</tr>
<tr>
<td><strong>Neutralising of pathogens</strong></td>
<td>recognition and eradication of plasmodia-infected red blood cells (13)</td>
<td>red blood cells, plasmodia</td>
<td>malaria</td>
</tr>
<tr>
<td></td>
<td>microbicidal activity of á-granule-derived á-defensins (107)</td>
<td>bacteria</td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td></td>
<td>entrapping of bacteria in cooperation with liver macrophages (14)</td>
<td>bacteria</td>
<td><em>Bacillus cereus</em>, (methicillin-resistant) <em>S. aureus</em></td>
</tr>
</tbody>
</table>


Other important adhesion molecules mediating cell-cell interactions are integrins and surface glycoproteins. Platelets express several \(\beta_1\) and \(\beta_3\) integrins, including integrins \(\alpha_5\beta_1\) and \(\alpha_2\beta_3\) (GPIIb/IIa) [reviewed in (168, 211)]. Integrin ligands on leukocytes are intercellular adhesion molecule (ICAM) and junctional adhesion molecules (JAMs) [reviewed in (45, 48, 212)]. Furthermore, GPIIb of the GPIb/IX/V receptor complex interacts directly with Mac-1 on monocytes and neutrophils (205) and GPVI mediates platelet recruitment and activation after collagen exposure facilitating secondary leukocyte capture [reviewed in (45)]. Increased PNC and PMC circulation was observed in patients with sepsis, atherosclerosis, inflammatory bowel disease, and cystic fibrosis (17, 44, 213) [reviewed in (44, 214)]. Finally, platelet-monocyte interactions are mediated and controlled by the stimulatory CD40/CD40L axis (126, 129) [reviewed in (129)].

During endotoxaemia, platelets tether and roll on endothelium and subendothelium via GPIIa and P-selectin, followed by GPIIb/IIa-mediated firm adhesion [reviewed in (10, 48, 212)]. This subsequently induces PSGL-1-mediated rolling of neutrophils on the P-selectin-expressing platelet layer. Neutrophil Mac-1 captures fibrinogen, which is presented by platelet GPIIb/IIa and GPIIa, resulting in neutrophil adhesion, a sequence of neutrophil recruitment that was recently deciphered in a murine model of encephalomyelitis (215). Accordingly, the infusion of lipopolysaccharide into humans induces PNC and PMC formation (216, 217). In summary, platelets closely interact with monocytes (218–220) and...
neutrophils (221) to regulate their recruitment to sites of inflam-
mation. In addition, PMC formation regulates the release of sev-
eral inflammatory mediators such as MDP-1, IL-8, TNF-α, IL-1β,
or MIP-1 [reviewed in (21)].

**NET formation**

Lipopolysaccharide (LPS)-activated platelets provoke the degran-
ulation of neutrophils (189). Plasma from septic patients activates
TLR4 on the platelet surface, inducing PNC formation, which cul-
minates in the release of NETs. Septic NET formation in mice oc-
curs primarily in liver and lung and supports pathogen clearance
by trapping (189) and finally neutralising of bacteria (222). Pla-
telet-neutrophil interactions ultimately further enhance phagocy-
tosis of pathogens (198). Triggering of NET formation by platelets
was found in murine models of bacterial and viral infection (223,
224). NETs have recently also been found in atherosclerotic
lesions, suggesting that NET formation may also be involved in
chronic inflammation (225). Indeed, the level of circulating NET
components is associated with advanced atherosclerosis in pa-
tsients with coronary artery disease (226). Finally, NETs and func-
tional leukocytes are an integral part of venous thrombosis, coin-
ing the expression “immunothrombosis” (2, 227). Patients with
thrombotic microangiopathies accordingly show increased levels
of circulating DNA and myeloperoxidase (228). NETs hence ap-
pear to be beneficial in severe bacterial infections, but may be an
attractive target for limiting immunothrombotic complications,
e.g. by degradation of extracellular chromatin.

**Pathogen capture and killing**

In addition to NET-mediated pathogen capture, platelets are also
able to bind and sequester bacteria directly in experimental mod-
els (8, 206): *In vitro*, platelets aggregate around bacteria entirely
“encapsulating” them to impede bacterial proliferation. HIV and *S.
aureus* were even documented to be internalised by activated pla-
telets (207). Both, HIV and *S. aureus*, were packed into engulfing
cavuoles that eventually fused with α-granules, possibly neutralis-
ing these pathogens. Platelets also aggregate around bacteria cap-
tured by Kupffer cells, facilitating pathogen neutralisation in a co-
operative effort between resident macrophages and recruited pla-
telets (14). Antimicrobial activity of platelets was also found in
murine malaria, where platelets bind *P. falciparum*-infected red
blood cells and release PF4 to kill the infected red blood cells to-
gether with the parasite (13). Similarly, platelet β-defensins directly
neutralise *S. aureus* (107). In mice, platelets bind Listeria monocyt-
togenes (and other gram-positive bacteria) via GPIb and comple-
ment C3 to facilitate the directed transport of the bacteria to DCs
in the spleen for clearance (116). Therefore, platelets not only
modulate immune functions of leukocytes but also contribute di-
rectly to the capture and in some cases even killing of pathogens. It
is unclear if these mechanisms can be exploited for patient treat-
ment.

**Potential future targets of platelet directed anti-inflammatory therapies**

Inhibitors of platelet adhesion receptors (e.g. GPIIb/IIIa inhibitors)
have been successfully developed in the past for the interference
with haemostatic/thrombotic platelet functions. Selectively target-
ing activated platelets with confirmation specific GPIIb/IIIa in-
hibitors was the next logical step (229). Advancement of this tech-
nology to produce inert (non-function blocking) activation-spe-
cific single-chain antibodies allows the targeting of activated pla-
telets and has successfully been used to enrich molecular contrast
agents at the site of inflammatory vascular injury and
(micro-)thrombosis (230–233) or to facilitate local fibrinolysis
(234, 235). The targeted inhibition – or enhancement – of immu-
nological platelet functions could be an attractive novel strategy
(236). Platelet TLRs are promising candidates for such a targeted
strategy. Of note, systemic targeting strategies (that bear the risk of
systemic adverse effects) are currently entering clinical phase II
trials with a TLR2 blocking antibody in IR injury (237).

**Conclusion**

Although the vast diversity of platelet immunity is well docu-
mented in animal or observational studies, it is surprising to note
that the pathophysiological relevance of platelet immune functions
is not well established in humans. Neither protection from autoim-
une disorders nor an increase in infectious complications has yet
been noted in the millions of cardiologic patients treated with pla-
telet inhibitors in clinical or post-marketing studies. Pre-clinical as
well as clinical trials are therefore needed to make a “clinical mean-
ing” of the diverse data summarized here, before patients can
benefit from this knowledge.
Conflicts of interest
None declared.

References


Duerschmied et al. Platelet immunity