Aspirin and P2Y$_{12}$ Inhibitors in platelet-mediated activation of neutrophils and monocytes

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Summary
Platelets are key players in haemostasis and represent a pivotal link between inflammation, immunity and atherogenesis. Depending on the (patho)physiological environment platelets modulate various leucocyte functions via release of inflammatory mediators and direct cell-cell interactions. Elevated levels of circulating platelet-leukocyte aggregates are found in patients suffering from various thrombotic or inflammatory conditions. Platelet-monocyte and platelet-neutrophil interaction can trigger pro- and anti-inflammatory responses and modulate effector functions of all leukocyte subpopulations. These platelet-mediated immune responses have implications for the progression of cardiovascular diseases and also play a crucial role during infections, cancer, transplantations and other inflammatory diseases of several organs. Antiplatelet therapy including the COX inhibitor aspirin and/or ADP receptor P2Y$_{12}$ inhibitors such as clopidogrel, prasugrel and ticagrelor are the therapy of choice for various cardiovascular complications. Both aspirin and P2Y$_{12}$ inhibitors attenuate platelet-leukocyte interactions, thereby also modulating immune responses. This may have beneficial effects in some pathological conditions, while it might be detrimental in others. This review aims to summarise the current knowledge on platelet-leukocyte interactions and the impact of aspirin and P2Y$_{12}$ inhibition on platelet-mediated immune responses and to give an overview on the effects of antiplatelet therapy on platelet-leukocyte interplay in various diseases.

Keywords
Platelet-leukocyte interaction, platelet immunology, inflammation, antiplatelet agents, aspirin, P2Y$_{12}$ inhibitors

Introduction
Platelets are central players in haemostasis. Upon injury or endothelial damage they become activated and rapidly aggregate to prevent blood loss. At the site of injury activated platelets trigger recruitment and activation of further platelets and leukocytes, thereby orchestrating immune responses and mediating wound repair processes (1, 2). Platelet activation triggers exocytosis of platelet granules, which comprise a plethora of immune-modulatory factors (2) (Figure 1A). Platelet dense granules are filled with adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin and calcium ions, which are important for activation and recruitment of further platelets. Platelet α-granules contain platelet factor 4 (PF4, CXCL4), macrophage inflammatory protein 1α (MIP-1α, CCL3), regulated on activation, normal T cell expressed and secreted (RANTES, CCL5), neutrophil activating protein 2 (NAP-2, CXCL7), interleukin 8 (IL-8) and IL-1β, CD40 ligand (CD40L) and P-selectin (CD62P), which are involved in recruitment and/or activation of leukocytes. Other α-granule factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), act on endothelial cells triggering wound repair processes (1, 3).

Activated platelets rapidly bind leukocytes. Their initial binding is mediated by platelet CD62P and leukocyte P-selectin glycoprotein ligand-1 (PSGL-1) (4). The affinity for CD62P varies between leukocyte subpopulations and is highest in monocytes, followed by granulocytes and lymphocytes (5, 6) (Figure 1B).

Platelet-leukocyte interactions are further stabilised by binding of CD40L to CD40 and via platelet glycoprotein GPIbα, junctional adhesion molecule-C (JAM-C) or fibrinogen bridging via activated GPIIb/IIIa with the CD11b/CD18 (Mac-1) integrin complex on leukocytes (7). Moreover, platelets interact with leukocytes via GPVI binding to extracellular matrix metalloproteinase inducer (EMMPRIN, CD147) or direct CD147-CD147 interaction (8), triggering receptor expressed on myeloid cells 1 (TREM-1)/TREM-1 ligand (9), CD15/CD62P and CD36/CD36 binding via bridging thrombospondin (7) (Figure 1A). Platelets can also...
communicate with leukocytes via microparticle release, which leads to receptor-mediated signalling, protein and nucleic acid transfer (10).

These direct interactions of platelets and leukocytes lead to targeted release of soluble mediators and mutual activation of both cell types, which is observed in various diseases. Platelet-leukocyte interplay fine-tunes immune responses and dependent on the (patho)physiological environment they can boost and dampen inflammatory responses. This makes platelets and antiplatelet agents an important but also dangerous player in various diseases.

Platelet-monocyte interactions and their effect on monocyte function

Among all leukocytes monocytes show the highest affinity for platelet CD62P and formation of platelet-monocyte aggregates is involved in a plethora of (patho)physiological processes. As depicted in Figure 2, platelets modulate monocyte functions in various ways. CD62P binding to monocyte PSGL-1 increases monocyte release of TNF-α, IL-1β, IL-6, IL-12 and IL-8, and platelet release of CCL5, CD40L and LIGHT boosts IL-6 and CCL2 expression in monocytes.

Figure 1: Direct and indirect platelet-leukocyte interactions. A) Platelet and leukocyte surface receptors and the most important platelet α-granule derived cytokines that modulate leukocyte responses are depicted. B) Differences in the affinity of leukocyte PSGL-1 to platelet CD62P. Fn, fibronogen; JAM-C, junctional adhesion molecule C; NK-cell, natural killer cell; PAF, platelet activating factor, PSGL-1, P-selectin glycoprotein ligand 1; TGF-β, transforming growth factor-beta; TREM-1, triggering receptor expressed on myeloid cells; TSP, thrombospondin.
monocytes (11–13), thereby accelerating inflammation. Platelet-derived CXCL4 boosts monocyte oxidative burst, which is further enhanced by platelet CCL5 (14). Platelet microparticles trigger monocyte activation, release of complement factor C5a and TNF-α secretion (15).

Among distinct monocyte subsets platelets preferentially bind to the CD16+ monocyte subpopulation (16), which is associated with various inflammatory diseases. In vitro platelets also trigger phenotypic changes towards intermediate and non-classical subsets by inducing CD16 expression (16, 17). As intermediate monocytes are known to secrete TNF-α and IL-1β (18), platelet-mediated CD16 upregulation may drive inflammation. However, the contribution of platelets to monocyte phenotype switch has not been investigated in clinical studies.

Platelet-monocyte interplay induces a pro-coagulant monocyte phenotype. Platelets induce monocyte tissue factor (TF) expression via direct cell-cell interaction and release of CXCL4 (19–21). Platelet-bound microparticles also display increased surface levels of coagulation factor FXa and fibrinogen (22). Taken together, platelet-monocyte interactions contribute to coagulation and might thereby play a role in both thrombosis and inflammation by limiting the dissemination of invading pathogens.

Upon platelet binding via CD62P and CD40L platelets deposit CXCL4 and CCL5 on endothelial cells and monocytes, leading to enhanced monocyte recruitment, adhesion and extravasation to sites of inflammation or injury (23, 24).

After recruitment to the vessel wall monocytes extravasate into the surrounding tissue and differentiate into macrophages, a process modulated by the presence of platelets. CXCL4 prevents monocyte apoptosis and induces their differentiation into macrophages (25). CXCL4-induced macrophages differ from classical M1 and alternatively activated M2 macrophages and are defined as M4 polarised macrophages (26). While polarisation of M1 and M2 macrophages is reversible, phenotypic changes into M4 macrophages are irreversible (27). M4 macrophages show decreased expression of scavenger receptors CD36 and SR-A, which leads to a reduced ability to take up oxidised low-density lipoproteins (oxLDL) (26). The phagocytic capacity of M4 macrophages seems to depend on the particles as one study reports only remnant phagocytic capacity (26), while another study revealed a high capacity for unspecific phagocytosis of CXCL4-stimulated macrophages (14). Evaluation of atherosclerotic lesions could recently confirm the in vivo existence and presence of M4 macrophages (28). However, their contribution to disease progression is currently unclear.

In contrast to CXCL4 alone, presence of platelets enhances lipid uptake and foam cell formation via cell-cell interactions, CXCL4 release and phagocytosis of lipid-laden platelets (16, 29) to promote the development and progression of atherosclerotic lesions.

Platelets also exert anti-inflammatory effects on monocytes: Activated platelets can diminish immune responses triggered by LPS, thyroglobulin or Porphyromonas gingivalis by enhancing production of IL-10 and suppressing TNF-α and IL-6 by monocytes (30). In a murine abdominal sepsis model functional loss of GPIIbα...
reduced circulating platelet-leukocyte aggregates but raised plasma levels of monocyte/macrophage-derived pro-inflammatory cytokines (31), indicating that platelets can diminish and accelerate inflammatory processes under pathological conditions.

**Platelet-neutrophil interactions and their effect on neutrophil function**

Similar to the platelet-monocyte interplay platelet interaction with neutrophils modulates neutrophil activation and function (Figure 3). Via soluble mediators and direct interaction activated platelets enforce neutrophil degranulation and release of pro-inflammatory IL-1β, IL-8 and matrix metalloprotease 9 (MMP9) (32, 33). Local release of platelet CXCL4, CCL3, CXCL7 and PAF (platelet activating factor) recruits neutrophils to sites of injury and inflammation (33–35). PAF and CXCL7 trigger neutrophil adhesion and spreading to the thrombus surface, where phosphatidylserine positive platelets bind neutrophils in preference to platelets (34, 35). Neutrophil interaction with platelets or platelet microparticles via CD62P, CD40L and GPIb induces expression, clustering and activation of Mac-1, which further stabilizes platelet leukocyte interactions (7, 33). Mac-1 and PSGL-1 binding to platelet counter-receptors augment mutual activation, adhesion and neutrophil extravasation (36), and Mac-1 activation also plays an important role in neutrophil phagocytosis (37).

Moreover, platelets indirectly enforce neutrophil extravasation by platelet-mediated activation of endothelial cells via direct interaction and/or soluble mediators (33). Platelet-derived serotonin is involved in endothelial activation in various inflammatory models (38). Platelet activation leads to CD40L shedding, which induces the formation of CXCL2, further augmenting Mac-1 expression, myeloperoxidase (MPO) release and neutrophil extravasation (39, 40). MPO, which catalyses hypochlorous acid production, is part of a host defense mechanism of neutrophils. Platelet-derived high-mobility-group-protein B1 (HMGB1) boosts MPO release, leading to oxidation of HMGB1, which increases neutrophil activation, triggering host defense mechanisms but also tissue damage (41). Generation of highly toxic reactive oxygen species (ROS), which provides another mechanism of neutrophils to kill invading pathogens, is enhanced by activated platelets (41).

On the contrary, platelet dense granule-derived ATP dampens MPO release and ROS production by neutrophils in endotoxaemia models (42), indicating that platelets are able to exert pro- and anti-oxidative actions, depending on the underlying pathophysiological condition.

In response to bacterial infections platelet interaction with neutrophils has been demonstrated to boost bacterial clearance. In severe sepsis neutrophils undergo a special form of apoptosis where neutrophils release their DNA content along with MPO, citrullinated histones and neutrophil elastase forming a web-like structure, which captures bacteria. This process is called neutrophil extracellular trap (NET) formation or NETosis. Activated platelets promote NET formation, resulting in increased bacterial clearance in vivo (32). Platelets further promote bacterial clearance by enforcing phagocytosis of bacteria (43) and surface exposure of phosphatidylserine in combination with CD62P results in phagocytosis of activated platelets themselves (44). Neutrophil...
mediated clearance of activated platelets might be an important mechanism to regulate inflammatory responses. Neutrophils can further diminish platelet-mediated inflammation by scavenging CCL3 and CCL5 via CCR5 during inflammation (45).

Taken together, many neutrophil-mediated host defense mechanisms, such as phagocytosis, ROS production and NET release, are stimulated by activated platelets indicating an important role of platelets for the elimination of pathogens.

Platelet-neutrophil aggregates not only enhance inflammatory responses but can also elicit anti-inflammatory effects by increasing generation of lipoxin A4, which downregulates neutrophil adhesion and extravasation (46).

Taken together, platelets seem to play a dual role in modulating neutrophil functions, which, depending on other environmental factors, exert pro- or anti-inflammatory functions.

**Platelet interactions with other leukocytes**

**Lymphocytes**

Lymphocytes also interact with activated platelets via platelet CD62P (Figure 1B), which results in modulation of lymphocyte function (Figure 4A). The initial interaction between platelet CD62P and lymphocyte PSGL-1 leads to clustering of CD11a and enhances leukocyte adhesion via intercellular adhesion molecule 1 (ICAM-1) binding and supports lymphocyte homing at the high endothelial venules of peripheral lymph nodes (47). Naive T-cells have a lower affinity to platelet CD62P compared to memory T-cells, which lack CD11a and therefore depend on platelet guidance to the peripheral lymph nodes (47). All lymphocyte populations are modulated by platelets and the same platelet factors can affect T-cell subtypes differently depending on the (patho)physiological microenvironment (6).

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**Platelet interactions with lymphocytes and dendritic cells.** A) Platelet interaction with lymphocytes enhances lymphocyte adhesion and extravasation. Platelet-lymphocyte interaction enhances homing, cytotoxicity and inhibits Th17 differentiation. Platelet interaction with B-lymphocytes enhances IgG switch and germinal centre formation. Natural killer cell interaction with platelets decreases their activation and alters tumour cell recognition. B) Platelet-dendritic cell interaction enhances dendritic cell recruitment, maturation and antigen presentation, but can also diminish dendritic cell activation and results in phagocytosis of platelets and platelet removal from preformed aggregates.
CD40 interaction with CD40L on the platelet surface or soluble CD40L is an important mediator of platelet-induced adaptive immune responses. CD40 is expressed on mature B-cells, some T-helper (Th) cells and cytotoxic T-lymphocytes as well as platelets (6). Via CD40L, platelets can directly induce B-cell antibody production (48) and support Th1-cell-mediated germinal centre formation (49). Antigen specific T-cells are rare and platelets can provide an alternative mechanism to boost B-cell responses. Platelet CD40L enhances cytotoxic T-lymphocyte activity and CD40L on Th cells can feed back to induce platelet activation (6).

Via CCL5 platelets augment T-cell adhesion on endothelial cells and facilitate their extravasation (6). Platelets further interfere with T-cell differentiation as CXCL4 has recently been shown to limit Th17 cell development (50).

Platelets also interact with natural killer (NK) cells and enhance NK cell extravasation at sites of injury but also pacify NK cell functions (6). Moreover, platelets adhere to and cover up tumour cells, thereby protecting them from NK cell-mediated lysis (6).

Dendritic cells

Platelets interact with dendritic cells via CD62P/PSGL-1 and subsequent Mac-1 binding. This interaction fosters the recruitment of dendritic cells to injured carotid arteries and facilitates their extravasation (51, 52).

Platelet interaction with dendritic cells could convey messages from sites of vascular lesions as this interaction caused dendritic cells to slow down and change their adhesive behaviour. Recruitment of dendritic cells to atherosclerotic lesions is increased in patients with acute coronary syndromes, and dendritic cells have been shown to be involved in the pathology of atherosclerosis by damaging the plaque structure (53). Platelet-dendritic cell interaction induces the maturation of IL-10 producing dendritic cells, which provoke allogeneic naïve T-lymphocyte proliferation with decreased IFN-γ production (54). Activated platelets enhance IFN-α secretion by immune complex-stimulated plasmacytoid dendritic cells through a CD40L/CD40 interaction (55). Other studies showed that CD40L on the platelet surface was not involved in dendritic cell maturation, neither are cell-cell contacts or plasma factors necessary (54, 56, 57). Thus, soluble factors excreted from activated platelets contribute to IL-10-producing dendritic cell maturation (54, 56).

Platelets interact with immature myeloid dendritic cells only under low shear conditions. Low shear rates are found in the microvasculature or at sites of reduced blood flow, e.g. around advanced atherosclerotic plaques or during atherothrombosis. Dendritic cells are shown to phagocytose activated platelets, thereby removing them from preformed aggregates (51, 52); this could be an important process under atherothrombotic conditions. Phagocytosis of platelets also results in JAM-C-mediated apoptosis of dendritic cells (52). The in vivo relevance of this finding is currently unclear, as atherosclerotic lesion progression coincides with platelet activation and dendritic cell extravasation. Platelet crosstalk with dendritic cells could trigger dendritic cell maturation and antigen uptake in the microvasculature or under atherothrombotic conditions.

Similar to platelet crosstalk with other leukocyte subpopulations, platelet-dendritic cell interactions are of dual nature as platelets can also diminish dendritic cell activation. In response to tissue damage cells release heat shock protein gp96 molecules, which is a dendritic cell activator. Platelets can neutralise gp96, which dampens dendritic cell responses (57). This might be important in conditions where wound-induced chronic inflammation and immune responses against auto-antigens have to be prevented. The effects of platelet interplay with dendritic cells are summarized in Figure 4B.

Effects of aspirin and P2Y12 antagonists on platelet-leukocyte crosstalk

Dual antiplatelet therapy comprising of aspirin and ADP receptor P2Y12 inhibition prevents thromboembolic events in patients with stable and unstable coronary disease and percutaneous coronary intervention and reduces the risk of overall deaths in these patients (58). Aspirin prevents prostanoid synthesis via inhibition of cyclooxygenase 1 (COX-1) and COX-2, thereby abolishing platelet production of TXA2, which represents an important mediator of platelet activation. Low dose aspirin almost exclusively attenuates COX-1 enzyme activity in platelets, while at higher dosage aspirin exerts anti-inflammatory effects by interfering with constitutive COX-1 and inducible COX-2 expression in various other cell types (59).

ADP represents an important physiological platelet agonist in haemostasis and thrombosis and activates platelets through purinergic receptors, P2Y1 and P2Y12. ADP-mediated platelet activation is an important positive feedback mechanism in response to various platelet agonists. Therefore, P2Y12-mediated platelet signalling also potentiates platelet activation induced by collagen, von Willebrand factor (vWF) and TXA2 (60).

Several studies confirmed that aspirin and P2Y12 antagonists reduce heterotypic platelet-leukocyte aggregates. Aspirin induced inhibition of TXA2 synthesis, systemically blocks CD40L release (61, 62) and reduces platelet-induced ROS formation, CXCL7 release, as well as platelet-mediated activation, recruitment, adhesion and extravasation of monocytes and neutrophils and diminishes foam cell formation (16, 63, 64). If these effects are merely a consequence of general platelet inhibition, or if antiplatelet therapies elicit platelet-specific anti-inflammatory effects aside from platelet aggregation is not clear. TXA2 generation does not appear to be important for activation of single platelets (65). Therefore the platelet inhibiting effect of aspirin is seen mainly as blocking of secondary platelet activation where cell-cell contact plays an important role. However, aspirin also seems to act as anticoagulant and counteracts vascular inflammation through mechanisms that do not involve platelet TXA2 formation. For example high doses of aspirin induce receptor shedding of platelets by COX1-independent pathways (66), thereby diminishing platelet activation, adhesion (67) and platelet interaction with other cells.
These off-target effects of aspirin might explain the increase in bleeding complications under aspirin intake, which does not occur in response to other COX1 inhibitors.

P2Y₁₂ signalling represents an important autocrine and paracrine feedback loop in response to most platelet agonists. Blocking this pathway also reduces autocrine activation of single platelets and might explain why P2Y₁₂ antagonists are more potent inhibitors of platelet-leukocyte aggregate formation compared to aspirin. Also P2Y₁₂ inhibitors elicit indirect effects on platelet function: Likewise, the ADP receptor antagonist clopidogrel improves endothelial nitric oxide bioavailability and suppresses platelet degranulation, platelet-leukocyte aggregate formation, expression of inflammatory cytokines and C-reactive protein (68, 69). In addition, P2Y₁₂ inhibitors reduce TF expression on monocytes (20, 70, 71), thereby antagonising the activation of the coagulation cascade.

Clopidogrel is more potent in reducing platelet-leukocyte aggregate formation in patients with atherosclerotic vascular disease compared to aspirin (72) and novel P2Y₁₂ inhibitors such as prasugrel show even stronger inhibition of pro-inflammatory effects of platelets (73). However, the benefits regarding ischaemic endpoints are balanced by increased risk of major bleeding in prasugrel compared to clopidogrel treated patients (74). Aspirin and P2Y₁₂ inhibitors also exert platelet-independent effects on leukocyte functions. Aspirin-induced COX inhibition enhances the generation of anti-inflammatory lipoxins, such as 15-epi-lipoxin, which has protective effects on post-ischemic hyperperfusion and lung injury (46, 75). P2Y₁₂ is also found on other cell types (76), and exerts non-platelet mediated pro- and anti-inflammatory effects on leukocytes (77, 78) and mediates leukocyte migration in transplant arteriosclerosis independently of platelet P2Y₁₂ (79). The effect of aspirin and P2Y₁₂ inhibitors on leukocyte functions further seems to depend on the pathological condition of the patient or on the disease models as effects observed in healthy volunteers are not always reflected by patient cohorts (80).

The role of platelet-leukocyte interactions and antiplatelet therapy in various diseases

Patients with stable and unstable coronary disease and those undergoing percutaneous coronary intervention are at major risk to develop thrombotic events due to enhanced platelet activation. Antiplatelet therapy comprising of aspirin and/or P2Y₁₂ antagonists are the therapy of choice for primary and secondary prevention of thromboembolic events in these patients (81). The anti-inflammatory effects of antiplatelet therapies in stable and unstable coronary disease are reviewed by Geisler et al. within this issue. Apparent clinical signs of coronary disease are often the result of a complex inflammatory response to multifaceted vascular pathologies over many years. Platelets are not only crucially involved in the atherothrombotic consequences of cardiovascular diseases (CVD) but also accelerate inflammation, thereby contributing to early steps of atherogenesis (23, 24). In line with these findings, several studies indicate that dual antiplatelet therapy including aspirin and ADP receptor inhibition not only prevents atherothrombotic events but also counteracts platelet-mediated acceleration of atherosclerotic lesion progression by interfering with platelet-leukocyte interactions in diseases beyond cardiovascular complications. Platelet-leukocyte interactions contribute to various diseases in multiple organs. Beneficial roles of aspirin and clopidogrel in these diseases are depicted in green, adverse effects are marked in red and no or neutral effects are marked in grey; COX-2, cyclooxygenase 2; NET, neutrophil extracellular trap; NK cell, natural killer cell; ROS, reactive oxygen species.
leukocyte interactions and subsequent leukocyte activation, infiltration, inflammation and lipid accumulation in atherosclerotic lesions (16, 27, 82). Platelets mediate leukocyte recruitment not only in CVD, but in multiple inflammatory conditions. Elevated levels of circulating platelet-leukocyte aggregates are found in patients suffering from various thrombotic or inflammatory conditions and serve as surrogate marker for platelet activation. The (patho)physiological consequences of these interactions in various diseases are discussed in the following chapter and summarized in Figure 5. Given the dual role of platelets under different pathophysiological conditions, dampening of platelet-leukocyte responses does not always improve clinical symptoms. Moreover, aspirin does not only affect platelet TxA2 synthesis but also modulates prostaglandin metabolism in various cell types. This can modulate direct and indirect effects on platelet-function and platelet-leukocyte interplay and thereby the course of disease.

Liver disease
Due to their pivotal role for vascular integrity, platelets are vital for normal liver function (83) and in response to injury they promote liver regeneration via serotonin release (84). However, in pathologic situations platelets and their interaction with leukocytes also seem to contribute to disease progression. Animal experiments revealed that platelet-leukocyte interactions play an important role in bile duct ligation-induced liver injury and that cholestasis-induced accumulation of platelets promotes leukocyte recruitment and worsens microvascular perfusion in a CD62P-dependent fashion (85). In a mouse model of chronic liver damage platelets enhance infiltration of neutrophils and cytotoxic T-lymphocytes into the liver via CXCL4 release (86), and in acute hepatitis platelet serotonin is responsible for impaired hepatic microcirculatory functions, disturbed virus clearance and cytotoxic T-lymphocyte-dependent liver cell damage (87, 88).

Antiplatelet drugs such as aspirin and clopidogrel inhibit the influx of cytotoxic T-lymphocytes and inflammatory leukocytes into liver tissue (88). Thereby, sustained administration of aspirin and clopidogrel not only reduces virus infection and liver damage, but also prevents hepatocellular carcinoma in mouse models of chronic hepatitis B virus infection (88, 89).

Renal disease
Glomerulonephritis, often caused by clearance of large quantities of immune complexes, leads to leukocyte activation and accumulation in the glomerular capillaries. Platelets enhance these processes via direct interaction with neutrophils and contribute to the pro-inflammatory environment accelerating renal damage (90).

Platelet adhesion and activation at sites of tissue damage boosts leukocyte recruitment but also triggers the initiation of tissue repair processes. Due to their pro-fibrotic function via release of TGF-β platelets play a pivotal role in the pathology of glomerulonephritis and glomerulosclerosis.

Clopidogrel has been shown to decrease TGF-β levels and to reduce glomerular matrix accumulation in an animal model of acute anti-thy1 glomerulonephritis (91) and to decrease leukocyte activation in renal failure patients (69). Prostaglandins are important for maintaining renal functions by counterbalancing the vasoconstrictive action of angiotensin II (92). Therefore, aspirin could have deleterious effects in glomerulonephritis and is contra-indicated in this disease.

Inflammatory bowel disease
Inflammatory bowel disease is associated with thrombocytosis and leukocytosis as well as an increased risk of thromboembolism (93). Patients with Crohn’s disease or ulcerative colitis show increased platelet surface expression of CD62P and CD40L and enhanced circulating platelet-leukocyte aggregates (93).

Elevated levels of IL-6 and thrombopoietin as well as iron deficiency have been suggested as the underlying mechanisms of increased platelet count and activation (93). Platelet activation and spontaneous aggregation is thought to occur in the mesenteric microcirculation, where platelets get exposed to inflammatory mediators, leading to their activation (93). Activated platelets then enforce the interaction between endothelial cells and leukocytes via direct interactions involving CD62P/PSGL-1 and CD40L/CD40 (94). Platelets further promote vascular permeability, enhance ROS production by neutrophils and enhance leukocyte infiltration into the mesenteric microcirculation (93). Neutrophils can drag platelets into the gut lumen, which triggers ATP/ADP/adenosine-dependent electrogenic chloride secretion and paracellular water movement, resulting in diarrhoea (95).

While P2Y12 inhibition by clopidogrel inhibited platelet activation and resolved symptoms of inflammatory bowel disease (96), the benefit of platelet inhibition by aspirin is unclear. A large scale study revealed no association of aspirin intake and inflammatory bowel disease (97), while other studies did find a strong positive correlation between Morbus Crohn’s disease and regular use of aspirin (98).

Skin disease, rheumatoid arthritis and systemic lupus erythematosus
Changes in platelet function and platelet-leukocyte interactions are involved in the pathogenesis of immediate and late-type hypersensitivity as well as chronic allergic inflammation and might contribute to the increased risk of cardiovascular events in these diseases.

Mouse models have demonstrated that activated platelets are capable of facilitating leukocyte rolling in cutaneous post-capillary venules and enhance the release of inflammatory mediators in the skin (99). Serotonin is crucial for the initiation of T-lymphocyte-mediated responses in the skin, such as contact sensitivity. CCL5 mediates the recruitment of eosinophils into the skin in allergen-induced late-phase skin reaction and dermatitis. Further, CD40L may induce dendritic cell maturation, enhance CD8+ T-cell responses and B-cell isotype switch in these diseases (99). In atopic eczema and dermatitis Staphylococcus aureus permanently colonises the inflamed skin, which enhances platelet-mediated
activation of immune cells via cell-to-cell contact of neutrophils and platelets. Platelets directly and indirectly recruit leukocytes into the inflamed tissue, thereby enhancing inflammation and preventing monocyte apoptosis (95, 100).

In a mouse model of immediate hypersensitivity reaction aspirin and clopidogrel were shown to reduce leukocyte extravasation by decreasing platelet-leukocyte interactions (101). However, aspirin can also potentiate acute allergies and cause adverse immunological reactions, like IgE-dependent mast cell activation, which further enhances adverse skin reactions (102).

In Raynaud’s phenomenon and systemic sclerosis platelet-leukocyte complex formation is enhanced but cannot be reversed by aspirin intake (103).

In psoriasis enhanced platelet activation results in neutrophil activation and elevated ROS formation and is also associated with an increased risk of CVD (83, 104). COX enzyme activity (99) and platelet CD62P expression were found to be enhanced in psoriatic patients and correlate with disease severity (105). Another large cohort study found no clear correlation between aspirin intake and the risk to develop psoriasis (106) whereas clopidogrel has been suggested as an exacerbating factor in psoriasis (107).

Approximately 30% of patients with psoriasis develop psoriatic arthritis. These patients, as well as patients with rheumatoid arthritis, antiphospholipid syndrome or systemic lupus erythematosus, show elevated circulating platelet-leukocyte aggregates (108, 109). However, the benefits of aspirin on platelet-leukocyte interactions in these diseases are still unclear as long term intake of aspirin by a small cohort of patients with antiphospholipid syndrome, rheumatoid arthritis or systemic lupus erythematosus had no effect on platelet activation parameters (109).

Transplantation
Platelet-leukocyte and -dendritic cell interplay contributes to vasculopathy and graft rejection in transplant patients. Activated platelets recruit leukocytes, alter their functions and augment leukocyte trafficking into targeted tissues (110). In a mouse cardiac transplant model platelets play a central role in maintaining CD4+ T_h cell homeostasis by regulating T_h differentiation via CXCL4 (50). Moreover CD40L/CD40 as well as CD62P/PSGL-1 interactions play a central role in platelet-mediated tissue rejection, transplant arteriosclerosis and allograft thrombosis as they promote inflammation and tissue damage as well as adverse adaptive immune responses. Antiplatelet therapy using aspirin has beneficial effects on platelet-mediated transplantation complications, while clopidogrel treatment shows no immune-regulating benefits (110).

Viral infections
Various viral infections coincide with platelet activation, platelet-leukocyte aggregate formation and increased platelet consumption, often resulting in thrombocytopenia. Platelet activation in viral infection is triggered via direct platelet-virus interactions as well as host immune responses. Platelets shape immune responses to many viruses and enhance chemotaxis and activation of various immune cells (111). CXCL4 suppresses human immunodeficiency virus (HIV) infection of T-lymphocytes (112) and platelets amplify adaptive immune responses via induction of T-lymphocyte-mediated germinal centre formation (49) and CD40L-mediated up-regulation of virus-specific IgGs (48). Platelets further recruit dendritic cells to sites of infection (52) and foster neutrophil activation and extravasation (113).

Virus-mediated platelet activation not only protects the host but may enhance viral infection since they are known to shelter viruses and support their dissemination by facilitating virus interactions with target cells (114).

Aspirin treatment has been shown to attenuate activation of platelets and leukocytes in HIV patients (115) and clopidogrel and aspirin have beneficial effects in animal models of hepatitis B infection (88).

Bacterial infections
Septic patients show elevated levels of circulating platelet-leukocyte aggregates and low platelet counts increase the mortality risk of septic patients (116). Platelet-leukocyte interactions are associated with enhanced inflammatory responses and improved detection and elimination of bacteria in early sepsis. Platelets enhance phagocytosis of bacteria (43) and provoke NET formation (32) but also augment pro-coagulatory processes (117). While platelet-neutrophil interactions accelerate inflammation and also induce tissue injury (116). In a murine endotoxemia model platelet interactions with macrophages inhibit macrophage release of inflammatory mediators, thereby counteracting inflammation and organ damage (118). Accordingly, in this model, aspirin treatment resulted in accelerated inflammation and decreased survival (118). On the contrary, clinical retrospective studies rather indicate a beneficial role of low-dose aspirin and/or clopidogrel on inflammation and prevention of organ failure (116). Moreover, patients taking aspirin and clopidogrel have lower incidences of pneumonia and bacteria-induced acute lung injury, despite being older and in a less healthy state compared to the control group (116). Taken together, aspirin and clopidogrel reportedly diminish inflammation and organ damage during bacterial infection. However, in cases of severe bacteraemia platelets might also dampen exacerbated inflammatory responses and aspirin treatment might have opposing effects. Further studies are necessary to fully understand the impact of antiplatelet agents during the course of bacterial infection.

Respiratory disorders
Platelets are critically involved in basal barrier integrity of the alveolar capillary endothelium and have specialised repair and remodeling activities in pulmonary, alveolar and bronchial vessels (119). Platelet interaction with leukocytes makes platelets a key player in acute lung injury (ALI), which is characterised by enhanced infiltration of neutrophils into the extravascular lung structures. Platelets contribute to tissue damage and severity of ALI via direct interaction with neutrophils and release of pro-
inflammatory mediators (120). TxA₂ release is a key mediator in ALI pathology and accordingly aspirin intake can prevent severity of the disease (120). High dose aspirin is superior to low-dose or dual antiplatelet therapy comprising of aspirin and clopidogrel in preventing ALI (121). The effect of aspirin does not only rely on diminished platelet pro-inflammatory cytokine release and platelet-leukocyte interplay, but also on the enhanced production of anti-inflammatory lipoxins (75, 120).

However, aspirin ingestion itself can trigger a chronic asthmatic disease termed aspirin exacerbated respiratory disease (AERD) via upregulation of cysteiny1 leukotrienes with oedema inducing properties. Platelet-leukocyte aggregates augment transeellular conversion of leukotrienes and a disturbance in platelet-leukocyte interactions e.g. by aspirin may be partly responsible for the respiratory tissue inflammation and the overproduction of cysteiny1 leukotrienes characterising AERD (122).

Cancer
Platelets influence tumourigenesis and metastasis through various mechanisms. Platelet-coated tumour cells are sheltered from elimination by immune cells (123) and platelet release of α-granules and synthesis of bioactive lipids further contribute to angiogenesis and tumour dissemination. Platelets induce leukocyte and tumour cell COX-2, which is an important regulator of tumourigenesis (59).

Continuous intake of low-dose aspirin, but not high-dose aspirin, lowered the long-term incidence of some cancer types (124). Aspirin diminishes platelet activity, thereby attenuating metastatic dissemination of cancer cells (124).

In experimental mouse models P2Y₁₂ deficiency or pharmacological inhibition significantly reduces metastases (125, 126) and hepatocellular carcinoma (89). However, in a small randomised control trial aspirin and clopidogrel had no effect on the number of circulating cancer cells in metastatic breast cancer (127). Further studies are warranted to estimate benefits of platelet inhibition by aspirin and P2Y₁₂ inhibitors for primary and secondary cancer prevention.

Conclusion
Platelet-mediated inflammatory responses are involved in a plethora of diseases. Thereby, inhibition of platelet activation by aspirin or P2Y₁₂ inhibitors has effects beyond regulation of haemostasis due to diminishing platelet-leukocyte interactions. This might indicate potential advantages of antiplatelet therapy under inflammatory conditions. However, inhibition of platelet function also bears an increased risk of bleeding events and unresolved effects during viral and bacterial infection. The effect of aspirin and P2Y₁₂ inhibitors further seems to depend on the dosage of antiplatelet drugs, pathologic condition of the study population, disease models or assays used. An exhaustive understanding of potential side effects of aspirin and P2Y₁₂ receptor antagonists is crucial to predict the clinical outcome in patients on antiplatelet therapy with additional medical conditions. Further large scale studies on the effects of antiplatelet therapy in inflammatory diseases are warranted to decipher their benefits or disadvantages in different pathological settings.

Conflicts of interest
None declared.

References