The Mutual Relation of Platelet Activation and Innate Immunity

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Abstract

Platelets are known to be central regulators of haemostasis, inflammation and immune response. Formed by megakaryocytes in the bone marrow and the lungs, platelets express a broad range of adhesion receptors *and release* cytokines and platelet microparticles which enable them to interact with both immune cells and pathogens. In bacterial and viral infections, thrombophilia and thrombocytopenia are commonly seen symptoms, indicating the close relationship between haemostasis and immune defence. Indeed, platelets contribute both directly and via immune mediation to pathogen clearance. In sterile inflammation, a pathogen-free process which is often triggered by cell necrosis and autoimmune reactions, platelets are also of central importance. Recently, platelet inflammasome has been extensively studied in this context. Both sterile inflammation and infection are affected by the interactions of platelets and innate immunity, notably the complement system. Although the general elements of this interplay have been known for long, more and more insights into disease-specific mechanisms could be gained recently. This review gives an outline of the current findings in the field of platelet–immune cell interactions and points out possible implications for clinical therapy.

Keywords
- platelets
- innate immunity
- inflammation
- infection
- cytokines
- inflammasome
- leukocytes
- complement system

Zusammenfassung

Thrombozyten sind bekanntlich zentrale Regulatoren von Blutstillung, Entzündung und Immunantwort. Gebildet von Megakaryozyten im Knochenmark und in der Lunge, exprimieren Plättchen eine breite Palette von Adhäsionsrezeptoren und setzen Cytokine und Plättchenmikropartikel frei, die es ihnen ermöglichen, sowohl mit Immunzellen als auch mit Pathogenen in Wechselwirkung zu treten. Bei bakteriellen und viralen Infektionen treten häufig Thrombophilie und Thrombozytopenie auf, was auf eine enge Beziehung zwischen Hämostase und Immunabwehr hinweist. In der Tat tragen Thrombozyten sowohl direkt als auch über die Immunmediation zur Pathogenclearance bei. Bei der sterilen Entzündung, einem pathogenfreien Prozess, der häufig durch Zellnekrose und Autoimmunreaktionen ausgelöst wird, sind Thrombozyten ebenfalls von zentraler Bedeutung. In letzter Zeit wurde das Thrombozyten-Inflammasom in diesem Zusammenhang ausführlich untersucht. Sowohl sterile Entzündungen als auch

Schlüsselwörter
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- angeborene Immunität
- Entzündung
- Infektion
- Zytokine
- Inflammasom
- Leukozyten
- Komplementsystem

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Infektionen werden durch die Wechselwirkungen von Blutplättchen und angeborener Immunität, insbesondere des Komplementsystems, beeinflusst. Obwohl die allgemeinen Elemente dieses Zusammenspiels seit langem bekannt sind, konnten in letzter Zeit mehr und mehr Einsichten in krankheitsspezifische Mechanismen gewonnen werden. Dieser Artikel gibt einen Überblick über die aktuellen Ergebnisse auf dem Gebiet der Wechselwirkungen zwischen Blutplättchen und Immunzellen und zeigt mögliche Implikationen für die klinische Therapie auf.

Introduction

Besides leukocytes and red blood cells, platelets constitute one of the three main blood cell types. They derive from megakaryocytes, large (50–100 µm in diameter), polyploid (up to 64N) cells which are formed in the bone marrow from hematopoietic stem cells. With an average diameter of 2 to 3 µm and a concentration of 150 to 450 $\times$ 10^9 per litre blood, platelets play an essential role in primary haemostasis, infection, modulation of immune response and tissue remodelling.[1–7] Although their different functions have been intensively studied, the process of platelet formation is recently attracting increasing interest as a detailed understanding of the factors, and mechanisms involved in thrombopoiesis can be of great use for the upcoming field of ex vivo platelet production.[8] In fact, efforts have been made to cultivate platelets for transfusions in the laboratory. Most attempts use megakaryocytes derived from experimentally generated human pluripotent stem cells to produce platelets in vitro.[9–11] Others follow the approach to infuse ex-vivo–produced megakaryocytes to stimulate platelet production in the lungs. However, low numbers of produced platelets and reduced platelet function are still obstacles to be overcome.[12] The *classical* process of megakaryopoiesis includes several steps from multipotent progenitor cells over bipotential megakaryocytic-erythroid progenitor cells to committed megakaryocytic progenitors cells.[13] This development is induced by thrombopoietin (TPO) and enhanced by the cytokines interleukin- (IL-) 3, 6, 9 and 11.[14–16] In a process referred to as *endomitosis*, megakaryocytes replicate their DNA several times without any cell division (►Fig. 1). As a result, several sets of chromosomes are present in megakaryocytes (between 4N and 64N).[17] Megakaryocytes in their late stages of development are then recruited to the bone marrow endothelial sinus by an interaction of SDF-1 with its CXCR4 receptor.[18] This classical model of MK migration has been recently challenged. Using an advanced combination of in vivo imaging techniques and computational simulations, Stegner et al could prove that the vast majority of MKs resided close to the blood vessels and showed very little migration. Furthermore, no differences could be observed in the localization of early- and late-stage MKs.[19] Thus, the principal theory that MKs migrate during their maturation from the osteoblastic to the vessel niche must be reconsidered.

However, recent reports indicated a second pathway of megakaryocyte formation. Indeed, Sanjuan-Pla et al demonstrated the existence of a platelet-biased subgroup of multipotent HSC which could be identified by the expression of megakaryocyte-related von Willebrand factor mRNA (vWF^+).[20] Another study determined that only a small subfraction of vWF^+-HSC showed coordinated megakaryocyte gene expression. These stem-like Mk-committed progenitors (SL-MkPs) belong phenotypically to the HSC compartment, but they are restricted to megakaryocyte lineage. While being in a quiescent state during homeostatic conditions, these cells are activated under inflammatory conditions and compensate the increased platelet consumption through a replenishment of the megakaryocytic progenitor cell pool.[21,22] In direct vicinity of the blood vessels, megakaryocytes form cytoplasmic processes ("pro-platelets"), which are connected to each other by cytoplasmic bridges. Furthermore, pro-platelets are elongated, branched and, guided by a gradient of sphingosine-1-phosphate (S1P), extended through endothelial gaps into the bloodstream.[23,24] Supported by the physiologic blood shear force, megakaryocytes release barbell-formed pro-platelets and their predecessors, discoid pre-platelets, into the blood system, where they rapidly separate into platelets.[13] Surprisingly, the last steps of platelet formation must not absolutely take place in the bone marrow. In fact, megakaryocytes and pro-platelets can also be found in the bloodstream and, as recently suggested, also in the lung sinus. Indeed, it is estimated that the percentage of platelets produced in the lung is as high as 50% of the total platelet count.[25]

Platelets and Infections

From everyday clinical experience, we know that the platelet count is altered in infections, autoimmune-mediated inflammation and disseminated intravascular coagulation (DIC).[26] These observations indicate the close relationship between platelets and immune cells in inflammatory processes and any host defence against bacterial and viral pathogens.[27,28]

Indeed, it becomes more and more evident that platelets play an important role in bacterial and viral infections, interacting both directly with pathogens and the responding immune cells. This interplay is not only relevant for basic science but also notably contributes to clinical pathologies. A recently published study indicates that acute respiratory infections with influenza types A and B or respiratory

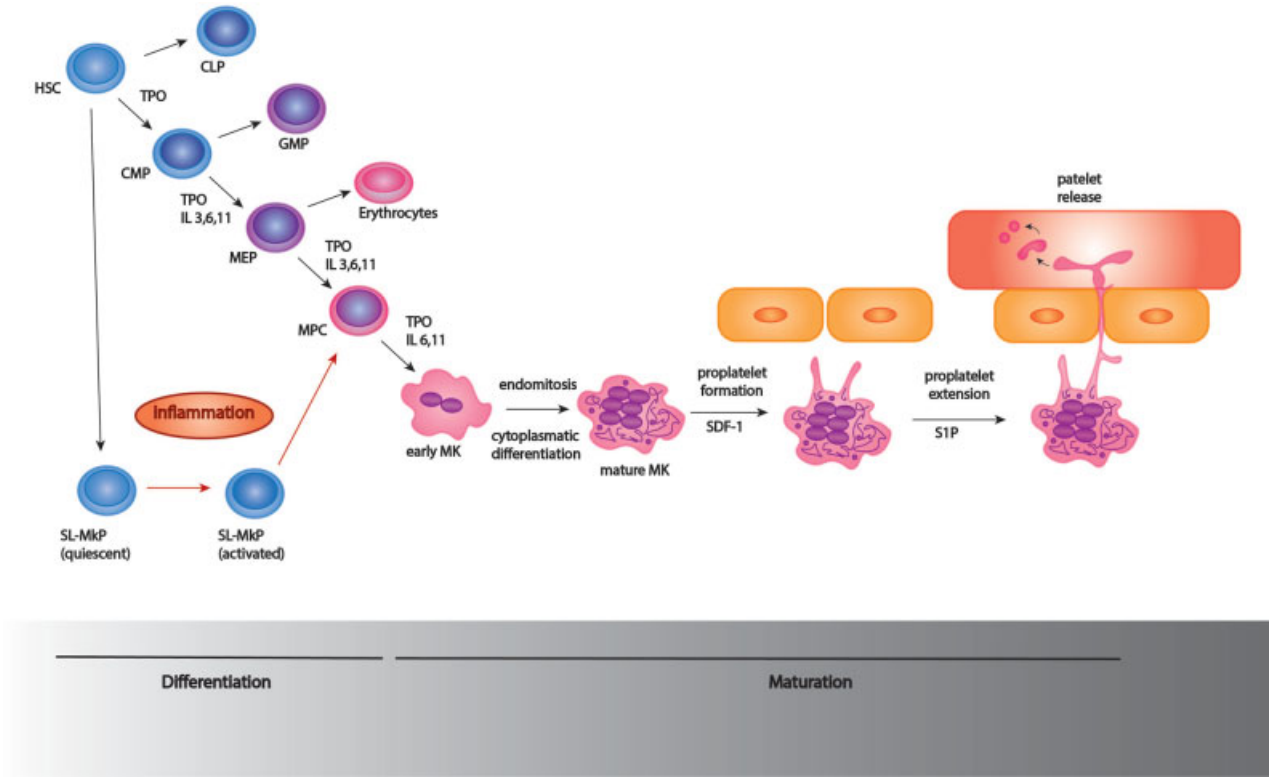

Fig. 1 Megakaryopoiesis and platelet production. Megakaryocytes are formed from pluripotent HSC, which under the influence of TPO develop into CMP cells and bipotential MEP cells. The latter further differentiates into committed MPCs that later form the early diploid megakaryocytes. During endomitosis, megakaryocytes become polyploid (up to 64N). The late stages of megakaryocytes are marked by the formation of pseudopodia *and their extension into the bloodstream*, where platelets are finally released from pro-platelets. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte–monocyte progenitors; HSC, human stem cells; MPCs, megakaryocyte progenitor cells; MEP, megakaryocyte–erythrocyte progenitor; MK, megakaryocytes; SDF-1, stromal cell-derived factor 1; SL-MkPs, stem-like Mk-committed progenitors; S1P, sphingosine-1-phosphate; TPO, thrombopoietin.

syncytial virus are strongly associated with the occurrence of myocardial infarction.[29] Indeed, during the first 3 days after laboratory detection of the viral infection, the incidence of myocardial infarctions was increased by six-fold compared with control. A possible explanation proposed by the authors of the study is an elevation of platelet activation due to the viral infection, which might give rise to an thrombogenic environment.[29] Congruently, a small study indicated a rise in platelet reactivity in blood samples from patients with viral respiratory tract infections compared with those of a control group.[30] Another explanation of increased troponin levels may, however, also be that a systemic reaction with increased stress to the body can aggravate any pre-existing coronary artery disease. Future studies will have to further scrutinize any direct links between infections, thrombosis, inflammation and coronary artery disease. Another study suggested that immune complexes formed during influenza A infection accounted for platelet activation.[31] In the context of bacterial infections, platelets were shown to be activated by binding of bacterial surface antigens to platelet receptors such as GPIbα,[32] GPIIb/IIIa[33] and toll-like receptor-2 (TLR2).[34,35] Although the specific activating ligands vary between the different bacterial strains, some studies suggested the binding of IgG-coated bacteria to platelet FcγRIIA receptor as a common pathway of platelet activation, also requiring the engagement of αIIbβ3.[36,37] A recently published study could prove that ATP-activated P2 × 1 receptor is an essential part of this activation pathway.[38] Platelets are activated by bacteria; however, they also contribute to the defence against bacteria in different ways. For instance, platelets were shown to encapsulate *Staphylococcus aureus* isolated from sepsis patients and to inhibit bacterial growth.[39] In a recent study, Gaertner et al described platelet migration as a novel mechanism of bacterial clearance.[40] This study demonstrated that platelets are able to migrate in an actin/myosin-dependent matter on surfaces coated with fibrin or fibrinogen in vitro and at the site of thrombus formation in vivo. It also indicated that platelets mechanically retracted parts of the surface they migrated on (►**Fig. 2**). Interestingly, migrating platelets were shown to form bundles with fibrin-bound bacteria, which led to neutrophil recruitment, phagocytosis by neutrophils and neutrophil extracellular traps (NET) formation.[40] Enhanced platelet–neutrophil interactions have also been reported after platelet stimulation with bacterial lipopolysaccharides (LPS).[41] In a model of LPS-induced sepsis, platelets stimulated the recruitment of neutrophils via serotonin secretion, which resulted in enhanced inflammation and impaired clinical outcome.[42] In fact, the role of platelets as a link between pathogens and immune cells becomes more and

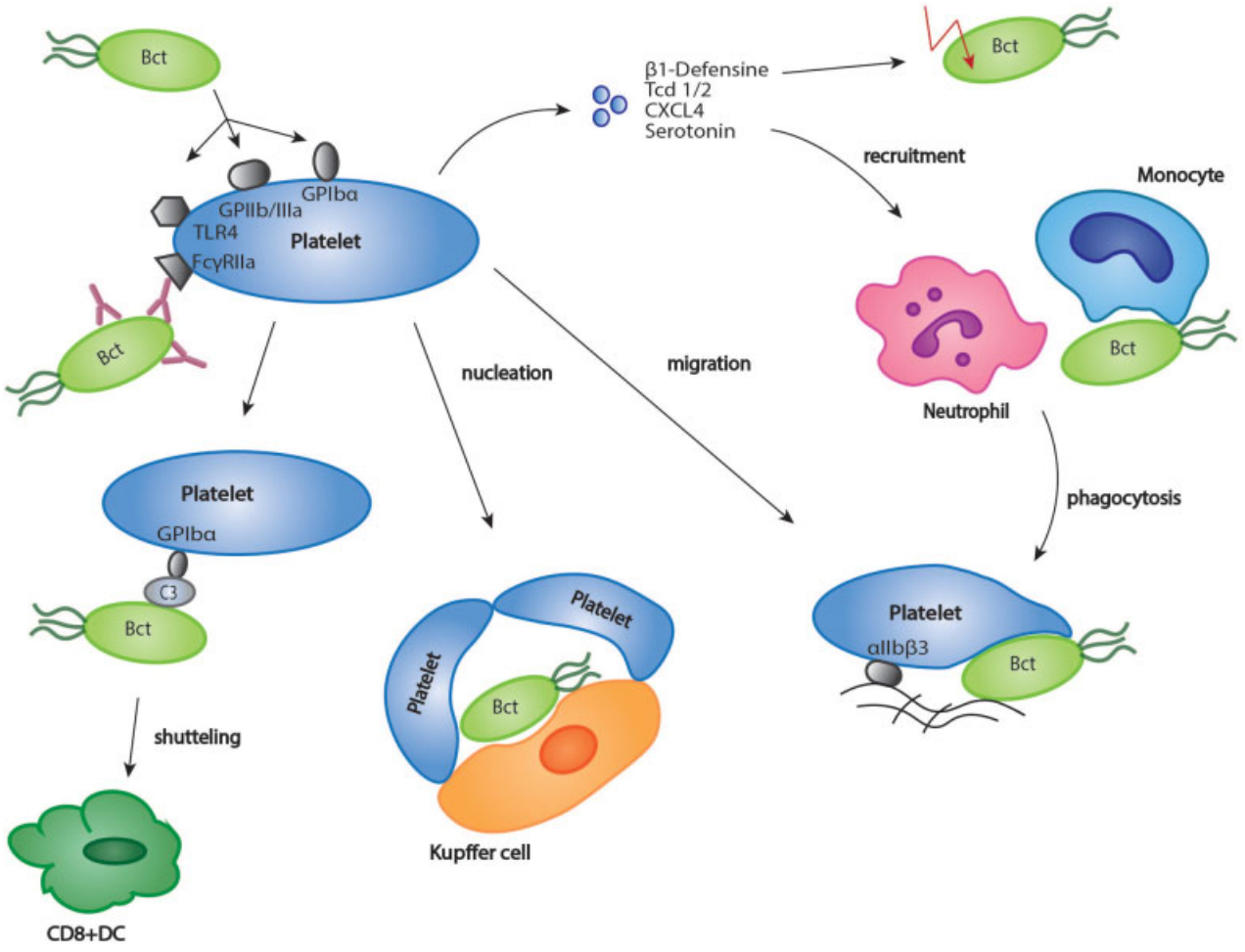

Fig. 2 Platelets as mediators of immune response in infections. Platelets can recognize bacterial surface antigens through adhesion receptors and pattern recognition receptors such as toll-like receptors. As a result, platelets secrete both microbicide peptides and chemokines that trigger innate immune response. Furthermore, they support pathogen clearance either by directing bacteria to phagocytic cells in the spleen or by enhancing liver macrophage (Kupffer cells) function. Recently, the role of platelet migration for immune defence has been underlined, as migrating platelets were able to encapsulate bacteria and to promote neutrophil response in the form of NET formation and phagocytosis. Especially in bacterial sepsis, platelets were shown to be essential for an adequate immune response. Bct, bacteria; C3, complement component 3; CXCL4 (PF4), chemokine (C-X-C motif) ligand 4; DC, dendritic cell; FcγRIIA, Fcγ-receptor IIA; GPIbα, glycoprotein Ibα; GPIIb/IIIa (αIIbß3), glycoprotein IIb/IIIa; Tcd 1/2, thrombocidin ½; TLR4, toll-like receptor 4.

more evident. Verschoor et al found that platelets recognized bacteria opsonized by the complement factor C3 via their GPIbα receptor, thereby promoting the shuttling of the complex to dendritic cells in the spleen.[28] This observation is in line with earlier studies that platelets can interact with antigen presenting DCs.[27] Indeed, activated platelets have been shown to induce the maturation of immature monocyte-derived DCs via release of CD 40 ligand.[43,44] Additionally, DC-induced lymphocyte proliferation was markedly enhanced in the presence of activated platelets.[27] Furthermore, platelets have been shown to recruit DCs through a MAC-1/JAM C and PSGL1/P-selectin dependent mechanism.[45] In line with this, platelets stimulated in vivo adhesion of DCs to an injured carotid vessel wall.[27] In an interesting translational study, Duffau et al indicated that platelets contributed to disease progression in systemic lupus erythematosus (SLE) via CD40L-induced activation of DC interferon-α secretion.[46] Thus, platelets contribute to maturation, recruitment and activation of dendritic cells. Furthermore, platelet-released PF4 was shown to attach to bacteria, which facilitated anti-PF4-antibody binding and thereby stimulated granulocyte phagocytosis.[47] Another study indicated that platelets enhance the uptake and intracellular killing of *S. aureus* by peritoneal macrophages, probably via a β1-defensin–dependent mechanism,[48] and that platelets are capable of directly killing bacteria, though the exact mechanism has not been further elucidated. However, other studies have already uncovered several microbicidal substances released by platelets, among them the proteins thrombocidin-1 and -2,[49] microbicidal chemokines termed *kinocidins* such as CXCL4[3,50] and the defensins β1 and β2.[39,51] Regarding these various platelet functions, the key role of platelets in defence against infections becomes evident. Indeed, a recent study by Wuescher et al underlined the importance of platelets for the clearance of bacterial infections. Using a model of diphtheria-toxin–induced conditional platelet depletion in

transgenic mice, this group found that platelet-depleted mice showed significantly reduced survival times in a *S. aureus*–induced context of sepsis and, thus, a severely enhanced bacterial burden.[52] In line with these results, Wong et al reported an essential role of platelets in the clearance of *Bacillus cereus* infection.[53] Interestingly, they could determine that platelets used their adhesion receptors GPIb and GPIIb/IIIa to encapsulate Kupffer's cells, intravascular liver macrophages, which have captured bacteria, thereby probably isolating the pathogens and supporting Kupffer's cell activity. In the same study, GPIb-deficient mice showed a 10-fold increase in liver cell death after infection compared with a control group.[53] In fact, in the context of acute and chronic liver infections, several studies demonstrated a remarkable influence of platelets on disease control. For instance, in the setting of viral hepatitis, platelet-released serotonin significantly decreased sinusoidal perfusion which impaired viral control and delayed cytotoxic $CD8^+$-T cell response, thereby promoting the development of a chronic viral hepatitis.[54] Furthermore, it was shown in hepatitis B virus pathogenesis that platelets adhere to liver sinusoids via CD44 and enabled the arrest of effector $CD8^+$-T cells that cleared infected hepatocytes by extending protrusions through endothelial gaps.[55] Concomitantly, another study demonstrated that, during acute viral hepatitis, platelets recruit cytotoxic T lymphocytes to the liver and, thus, contributed to progression of inflammation and liver damage.[56] Platelets are an important element in the host defence against the malaria–causing parasite *Plasmodium falciparum*. Actually, in a model of malaria infection, platelet-deficient mice showed higher parasitaemia levels and higher mortality rates than control animals.[57] Unexpectedly, it was shown that platelet-derived PF4 could be internalized by parasite-infected red blood cells via their Duffy antigen receptor for chemokines.[58] Once in contact with the intracellular parasite, PF4 induced the lysis of the parasite digestive vacuolar membrane, which led to a rapid elimination of the parasite.[59] In reaction to these results, the use of anti-platelet drugs in clinical treatment of malaria patients was critically discussed.[60] However, a recent study could neither find any effect of platelet depletion on parasitaemia nor find a direct elimination of intraerythrocytic parasites by platelets in vitro.[61] Hence, future studies will have to elucidate the role of platelets in malaria infection.

Furthermore, platelets might also become themselves a target of viral pathogens. In the case of dengue fever, patients often suffer from severe thrombocytopenia and hemorrhages.[62] In an elegant study, Simon et al could prove that dengue virus directly adheres to platelet receptors and—through a yet unknown mechanism—invades the cells, where they stimulate the production and release of infectious viral particles.[63] Immune recognition of platelet-bound viral antigens might contribute to an augmented platelet clearance and thrombocytopenia. Interestingly, the same study also demonstrated that binding of dengue virus was markedly enhanced in thrombin-activated platelets. Congruently, Ojha et al found that platelet activation was correlated with low platelet counts in dengue patients and that activated platelets showed high numbers of copies of dengue virus genome.[64] Another severe manifestation of dengue fever is the capillary leakage syndrome due to an increased vascular permeability. It could be shown that platelet exposition to dengue virus led to a rise of mitochondrial reactive oxygen species production in platelets, which triggered platelet inflammasome activation and IL-1β secretion, the latter accounting for the increase in endothelial permeability.[65]

In general, elucidating the mechanisms of platelet activation and platelet response to bacterial and viral infections might help complete our understanding of severe diseases such as infective endocarditis (IE), DIC in sepsis or viral hepatitis. In IE, low platelet counts have been associated with increased 6-month mortality.[66] Furthermore, in a model of experimental *S. aureus* endocarditis, bacterial susceptibility to thrombin-induced platelet microbicidal protein determined disease progression markers such as bacteraemia and valvular tissue damage.[67] Therefore, platelets seem to play an important role in the clearance of IE. However, a recent study found that platelets also contributed to NET-dependent bacterial biofilm formation on injured heart valves in a Streptococcus mutans endocarditis model.[68] Thus, platelets have both beneficial and detrimental effects on IE modulation. DIC is marked by extensive platelet activation and microvascular thrombosis.[69] Recently, several studies were able to demonstrate that platelets bound to neutrophils during sepsis and stimulated the secretion of procoagulant NET, which promoted intravascular coagulation.[41,70]

In conclusion, platelets have several strategies to cope with invading pathogens. They release microbicidal substances, bind and isolate the pathogen, and recruit phagocytic immune cells. Although these reactions usually contribute to pathogen clearance, platelets also have been shown to aggravate diseases, for instance viral hepatitis, dengue fever or DIC.

Platelets and Sterile Inflammation

The role of platelets in inflammation has been well investigated. Platelets use a variety of receptors (CD40L, P-selectin) and cytokines (PF4, RANTES, IL1β) to interact with leukocytes such as granulocytes, lymphocytes, monocytes and dendritic cells.[2] Indeed, one key role of platelets is the recruitment of phagocytic cells to lesion sites. Exposing P-selectin on their surface, vessel-bound platelets slow down monocytes and neutrophils at vascular lesions and then establish firm adhesion to them via a CD11/18 (Mac1)-dependent mechanism.[71,72] Besides the sole recruitment of immune cells, platelets also contribute to leukocyte activation. For instance, the P-selectin–dependent binding of platelets to monocytes resulted in an upregulation of monocyte activation markers.[73] In neutrophils, platelet P-selectin could be proved to induce cell activation and release of NET.[74] Furthermore, the platelet-released chemokines RANTES and PF4 significantly stimulated the arrest of phagocytic cells on activated endothelium.[75] Surprisingly, coculture of platelets with T lymphocytes led to decreased INFγ/TNFα production and reduced lymphocyte activation.[76]

Thus, platelets seem to both enhance and regulate immune cell reaction.

The process of inflammation is not only essential for our immune defence in microbial infections, but it also contributes to the induction of repair mechanisms and tissue regeneration after mechanical or chemical tissue injury.[77] This sterile, pathogen-independent inflammation plays a central role in the genesis of a surprisingly broad spectrum of diseases. Indeed, post-ischemic reperfusion (myocardial infarct, stroke and acute renal injury), mechanical trauma, crystal depositions (gout, pseudogout, silicosis and atherosclerosis), particles (asbestosis) and tumour cells can be found among the triggers of sterile inflammation.[78–81] In these often chronic diseases, inflammation rather promotes disease progression than preventing it, which makes it an interesting target for clinical therapies (►Fig. 3). In general, sterile inflammation is induced by necrosis, an uncontrolled form of cell death, which leads to the extravasation of proinflammatory cell contents such as ATP, mtDNA (from mitochondria), uric acid, heat shock proteins (HSP) and S100 proteins from the cytosol as well as HMGB1, histones and DNA from the nucleus.[82] Referring to the pathogen-associated molecular patterns (PAMPs) expressed on microbes, this group of inflammation-inducing cellular components has been termed *danger-associated molecular patterns (DAMPs)*. ATP can be released from the mitochondria of both apoptotic and necrotic cells. During apoptosis, ATP secretion is mediated by pannexin-1 channels and serves as chemoattractant signal for phagocytic monocytes and macrophages.[83,84] Interestingly, a recent study could demonstrate that extracellular ATP stimulated an autocrine pannexin-1-channel–dependent loop in dendritic cells, which enhanced their migration to draining lymph nodes.[85] Necrotic cell ATP release has been determined to significantly induce NLP3 inflammasome activation and subsequent IL1β secretion.[86,87] Thus, ATP is essential for inflammatory cytokine release and leukocyte migration in sterile inflammation.

Uric acid is produced through enzymatic degradation of purinergic nucleotides both in intact and dying cells. However, cell necrosis leads to the extracellular release of uric acid, where it has been shown to constitute one of the major inductors of sterile inflammation.[88] HSP, and most important

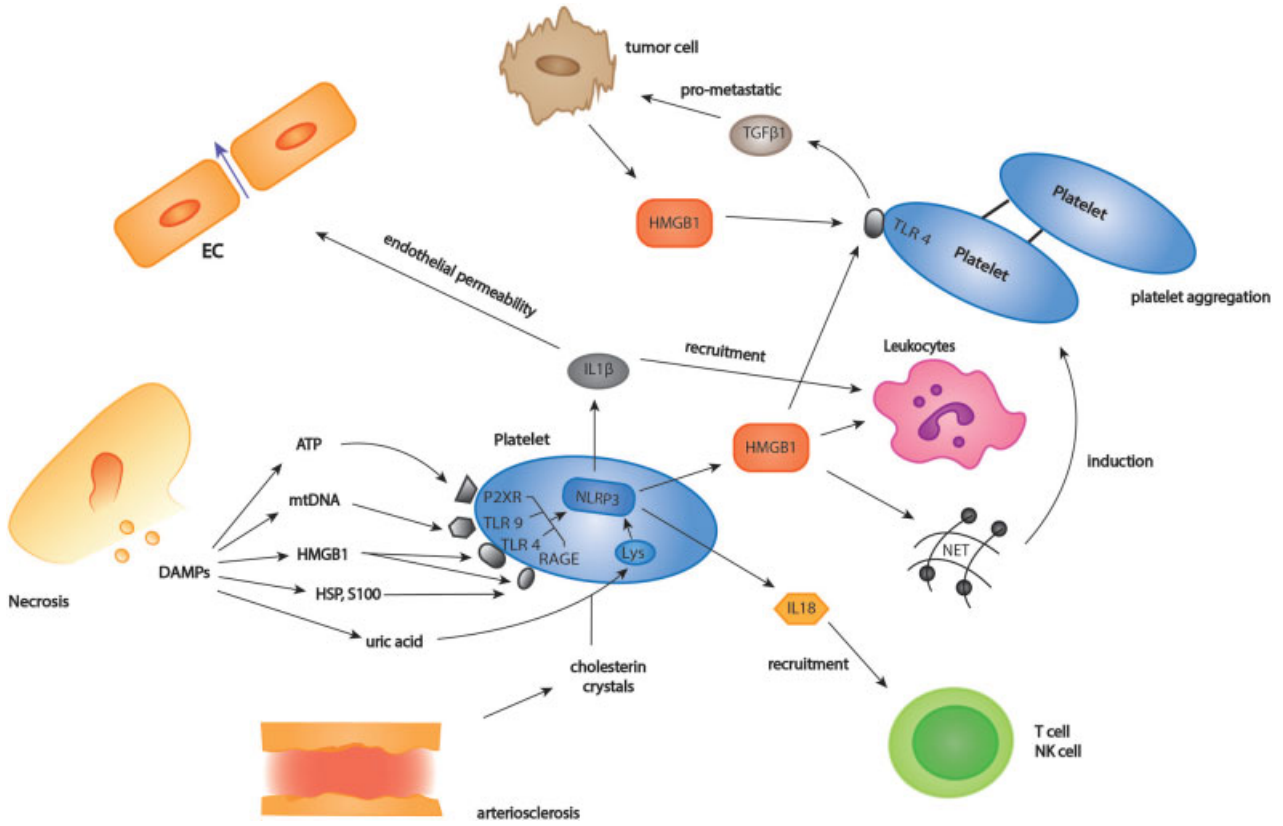

Fig. 3 Regulation of sterile inflammation by platelets via multiple inflammasome-dependent mechanisms. Sterile inflammation can be caused by the uncontrolled release of cellular components during the process of cell necrosis. It contributes to the progression of autoimmune diseases, cardiovascular diseases and tumour growth. Platelets expose different pattern recognition receptors which can detect damage-associated molecular patterns released during cell injury such as heat shock proteins and DNA. Most of them trigger the activation of platelet inflammasome, which in turn promotes the release of proinflammatory cytokines. Especially platelet HMBG1 was shown to play an essential role in immune cell recruitment, neutrophil response but also thrombus formation. Thus, it might be a potential target in inflammatory thrombotic diseases such as arteriosclerosis and myocardial infarction. Other platelet-released cytokines, among them interleukin 1β and 18, enhance endothelial cell permeability and adaptive immune cell response, which further drives inflammation and disease progression. DAMP, danger-associated molecular pattern; EC, endothelial cell; IL 1β/18, interleukin 1β/18; HMGB1, high-mobility group protein B1; HSP, heat shock protein; Lys, lysosome; NET, neutrophil extracellular traps; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3; P2XR, P2X receptor; RAGE, receptor for advanced glycation end products; TGFβ1, tumour growth factor β1; TLR4/9, toll-like receptor 4/9.

HSP 70, can also be found among the mediators released by necrotic cells.[89] Several studies indicated that members of the HSP 70 family triggered dendritic cell activation and consecutive T lymphocyte response, which might even result in the development of autoimmune diseases.[90,91] HMGB1 is a component of the cell nucleus and can be released during necrosis, but not apoptosis.[92] Furthermore, it promotes inflammatory reaction and elevated HMGB1 levels have been found in many inflammatory and autoimmune diseases.[93,94] Other elements of the DAMP group are nucleus-derived extracellular histones. Indeed, these nuclear proteins activate TLR2/4 signaling and subsequently induce sterile inflammation, especially when they are bound to DNA.[95] Recently, Kawai et al found that injection of histones led to dose-dependent multiple organ injury in mice, which could be reduced by anti-HMGB1 treatment.[96] Extracellular DNA released from the cell nucleus is present both in necrotic and apoptotic cell death.[97] Although bacterial DNA has been identified as a ligand of PAMP-associated TLR9 receptor, isolated endogenous DNA did not provoke an inflammatory response.[98,99] However, Urbonaviciute et al demonstrated that endogenous DNA from apoptotic cells formed complexes with HMGB1, which have been able to activate antigen presenting cells and to trigger cytokine release.[100] DAMPs may also be derived from extracellular sources, especially during extracellular matrix degradation.[80] Indeed, hyaluronan fragments produced during ECM degradation in acute lung injury have been shown to initiate an inflammatory response in antigen-presenting cells through a TLR2/4-dependent mechanism.[80]

Immune cells are able to recognize DAMPs via extracellular (TLR2/4, C-type lectin receptors [CLR], receptor for advanced glycation end products [RAGE]) and intracellular (NOD-like receptor [NLR], absent in melanoma 2 [AIM2]) receptors of the pattern recognition receptor (PRR) family.[101–103] Several receptors contribute to the recognition of DAMPs. Toll-like receptors 2 and 4 recognize microbial membrane components such as LPS and also endogenous danger-associated molecules, among them are extracellular histones and HMGB1.[104] Therefore, they are powerful regulators of both sterile and infection-triggered inflammation. For instance, recent studies indicated a major contribution of TLR2/4 to the auto-inflammatory processes of hyperoxia-induced retinal vessel regression or human male infertility caused by sterile inflammation.[105,106]

During the last decade, the CLR family has come into the focus of research on sterile inflammation receptors. In fact, members of this family have been shown to sense DAMPs such as uric acid, F-actin and SAP130 and thus effectively detect necrotic cells.[107–109] Interestingly, activation of CLRs by cell death released SAP130-induced inflammation and neutrophil recruitment, whereas stimulation by uric acid markedly reduced neutrophil activation and inhibited inflammation.[107,109] Hence, CLR family shows complementary effects on sterile inflammation.

RAGE acts as a receptor for various DAMPs, among them are HMGB1, S100 and amyloid β.[110,111] Furthermore, the role of RAGE in sterile inflammation is well established. For instance, RAGE-induced inflammatory response inhibited hepatocyte regeneration after massive liver injury.[112] In a model of myocardial ischemia, RAGE significantly enhanced ischemia/reperfusion injury.[113] Recently, Bangert et al uncovered that the interplay of HMGB1 and RAGE essentially contributed to autoimmune myocarditis and inflammatory cardiomyopathy, thereby indicating the receptor as a novel therapeutic target.[114]

Nod-like receptors such as NLRP 3 and AIM2 are key players of sterile inflammation, since they form, together with an adaptor protein (ASC) and caspase-1, a complex called *inflammasome*, which upon activation stimulates secretion of the proinflammatory cytokines IL-1β, IL18 and HMGB1.[115,116] Indeed, NLRP 3 inflammasome stimulation by cholesterol crystals has been proved to significantly induce arteriosclerotic plaque formation.[117] AIM2 has been originally identified as a receptor for cytosolic DNA.[103] However, recent studies indicated that AIM2 inflammasome also plays a key role in the induction of sterile inflammation, for instance after acute ischemic brain injury.[118]

In addition to classic immune cells, platelets were shown to contribute to sterile inflammation.[119] Indeed, platelets express PRRs such as TLR2, 4 and 9, which are known to sense DAMPs released during tissue injury.[120,121] Interestingly, Yu et al could demonstrate that tumour cells activate platelet TLR4 via secretion of the proinflammatory HMGB1, thereby inducing platelet recruitment and platelet release of the metastasis-promoting factor TGFβ1.[122] The physiologic mechanisms involved in HMGB1-induced cell activation have not been well understood until recently. However, recently it was uncovered that in a model of retinal ischemic reperfusion injury, HMGB1 binding led to the activation of NLPR3 inflammasome and consequently to the release of IL-1β.[123] Concomitantly, platelets were shown to release the proinflammatory IL18 through an inflammasome-dependent mechanism.[124] Thus, platelets seem to be part of the sterile inflammatory process. Remarkably, it is well documented that platelets are able to secrete HMGB1 upon activation.[125,126] As HGMB1 has a chemoattractant and cytokine-stimulating effect on leukocytes, this indicates a possible link between platelets and immune cell regulation.[127] Indeed, platelets can attract monocytes via a HMGB1-TLR4–dependent pathway and promote downregulation of monocyte apoptosis through the interaction of platelet HMGB1 with monocyte RAGE.[128] Furthermore, binding of platelet-derived HMBG1 to neutrophil RAGE was shown to trigger the release of NETs.[129] However, HMGB1 has not only an effect on leukocyte recruitment but also influences platelet activation and thrombosis. For instance, HMGB1 leads to the activation and aggregation of platelets by ligation of platelet TLR4, but not TLR2 and RAGE.[130] Interestingly, another study using activated platelets indicated that HMGB1 interacts primarily with platelet RAGE, but also documented increased levels of HMGB1 in coronary artery thrombi.[131] In line with this, platelet HMGB1 has the ability to increase thrombus formation (predominantly via TLR4).[132] In models of $FeCl_3$-induced mesenteric artery thrombosis and trauma/haemorrhagic shock,

deficiency of platelet HMGB1 led to prolonged thrombus formation times and reduced small vessel thrombosis. Additionally, loss of platelet HMGB1 significantly diminished inflammatory reaction after trauma in the form of NET formation and proinflammatory cytokine levels.[132] In a model of deep vein thrombosis, platelets were identified as the most important source of HMGB1, thus contributing essentially to thrombus formation, leukocyte recruitment and immune cell activation.[133] Closing the circle, a recent study demonstrated that platelet inflammasome, among others, a down-stream effector of the HMGB1 signaling cascade, plays an important role in platelet activation and aggregation.[134] Another recently published study even linked the effects of HMGB1 on immune cells and thrombus formation. Using a model of deep vein thrombosis, Dyer et al confirmed the prothrombotic and NET-stimulatory effect of platelet HMGB1. In addition, this group showed that inhibition of NET formation significantly impaired the effect of HMGB1 on thrombus formation, which hints at a neutrophil-dependent mechanism of deep vein thrombosis.[135] In a clinical approach, a recent study proved that aspirin therapy in high cardiovascular risk patients diminished expression of HMGB1 in platelets, indicating another mechanism of aspirin drug efficacy in cardiovascular diseases.[136] Further studies have to further question whether platelets could be used as possible targets for therapies in sterile inflammatory diseases.

Summing up, the role of platelets in sterile inflammation has to be added to our common knowledge of platelet–immune cell interactions. DAMPs are released during both necrotic and apoptotic cell death and can be sensed by platelet PRRs. Subsequently, platelet inflammasome activation leads to the release of inflammatory mediators such as HMGB1, which introduce the activation of immune cells and stimulate thrombus formation via neutrophil-dependent NET secretion. These new insights into platelet functions extend the range of platelet-mediated diseases from autoimmune diseases to post-ischemic injury.

Platelets and Cancer

Platelets are not only an important element of the immune response against infectious microorganisms, but also modulate tumour development. In a recent study, elevated platelet levels have been identified as a strong risk marker for cancer, especially in male patients.[137] Furthermore, malignant neoplasms are often associated with an elevated risk for thrombosis and thrombophlebitis.[138] In fact, cancer cells stimulate platelet activation through the release of potent mediators such as HMGB1, tissue factor, ADP and thromboxane.[122,139–142] Activated platelets adhere to cancer cells in the bloodstream and facilitate their arrest and migration through the vessel wall, thereby promoting tumour metastasis.[143,144] In addition, the activated platelets shield metastatic cancer cells from immune cell recognition and NK-mediated cell lysis.[145] Recently, platelets have been also shown to suppress T cell response against cancer cells via TGF-β.[146] Finally, platelet granules contain pro- and antiangiogenic factors, which are released upon tumour-dependent platelet activation.[147] However, their influence on tumour angiogenesis has not been fully understood yet.

Platelets and Platelet Microparticles

Microparticles (MP) are defined as plasma membrane vesicles with an average size of 0.1 to 1 µm, which can be released by a broad variety of cells during activation, cell stress or apoptosis. Although leukocytes, endothelial cells, erythrocytes and megakaryocytes are also known to shed MPs, platelets constitute the major source (70–90%) of MPs in the bloodstream. Platelet microparticles (PMPs) have been shown to contribute both to physiological and pathological processes.[148] For instance, during haemostatic clot formation, PMPs significantly enhanced fibrin clot stability, whereas lack of PMPs strongly prolonged the time required for thrombin generation.[149] Indeed, patients with a deficiency in PMP production (Castaman's syndrome) suffer from prolonged bleeding times and haemorrhages.[150] Interestingly, Ponomareva et al determined that PMPs differed in size, structure and density according to the platelet-activating stimulus.[151] In line with this, a recent study showed that a subgroup of PMPs contains mitochondria and that mitochondrial membrane degradation by soluble phospholipase A2 provoked an inflammatory response.[152] Another study demonstrated that PMPs stimulated inflammation in rheumatoid arthritis, probably via IL1-mediated activation of fibroblast-like synoviocytes.[153] In cancer patients, elevated PMP levels have been associated with metastasis and higher tumour aggression.[154] However, a recent study indicated that PMPs were able to transfer miRNA into solid tumour cells, which led to tumour cell apoptosis and inhibition of tumour growth.[155] Thus, the role of PMP in cancer progression remains to be clarified, yet. Moreover, PMPs might also influence tissue regeneration. For instance, platelet-derived MPs have been shown to induce proliferation and tube formation in human umbilical vein endothelial cells (HUVEC) in vitro.[156] Furthermore, PMP injections significantly enhanced angiogenesis in an in vivo model of chronic myocardial ischemia.[157] Together, platelet-derived particles are complex mediators of processes such as inflammation, cancer progression and tissue regeneration and will be of great interest to future research.

Crosstalk Platelets—Complement

The name of the complement system already anticipates its functions—indeed, with more than 30 enzymes and inactivated precursor proteins involved, the complex cascade contributes to the amplification of most immune responses to invading pathogens and promotes tissue regeneration and cell clearance after injury.[158,159] It is therefore not surprising that platelets also interact with parts of the complement system in various physiological and pathological processes. In general, complement activation can be triggered either by surface-bound IgG and IgM antibodies via the C1qrs complex (classical

pathway) or by bacterial carbohydrate antigens (MBL-MASPs–dependent lectin pathway) and bacterial LPS (via C3bB in the alternative pathway).[160] All three pathways result in a common trunk, in which the most abundant complement C3 is cleaved into the chemoattractant anaphylatoxin C3a and the opsonizing C3b. Furthermore, C5 is cleaved into C5a, another anaphylatoxin, and C5b, which together with C6, 7, 8 and 9 forms the cell lysis inducing membrane attack complex (MAC). The complement components are in close contact with cells of innate and adaptive immunity.[161,162] Activated platelets have been shown to influence this complex network by contributing to complement activation.[163] For instance, activated platelets were able to bind properdin, a positive regulator of alternative pathway activation, and thereby promote the formation of alternative pathway convertase (C3bBb and C3(H2O)Bb) on their surface, which further stimulated the complement cascade.[164] Concomitantly, another study suggested that platelet P-selectin serves as a receptor for C3b, thus initiating the formation of C3-convertase and of the MAC on platelets.[163] In addition, it was shown that platelets secreted chondroitin sulfate that bound C1q and activated classical complement pathway.[165] Further amplification of the classical pathway might be effectuated by the exposure of negatively charged molecules such as phosphatidylserine on the surface of platelets and platelet-derived MPs.[166–168] Yet, platelets are able to suppress complement activation via the release of C1 inhibitors.[169] Interestingly, it could be demonstrated that during low-shear stress, platelets rather promoted complement activation, whereas high stress led to enhanced production of complement-inhibiting factors.[170] Besides these platelet effects on complement activation, complement elements influence platelets vice versa (►Fig. 4). In fact, platelet activation and aggregation can be triggered by complement factors.[171,172] The complement components C1q, C3, C4 and C9 are able to bind to the surface of activated platelets, respectively.[173] In line with this, platelets were shown to express C1q receptors on their surface and C1q multimers triggered platelet activation and aggregation.[174,175] Furthermore, platelet–neutrophil interactions were significantly decreased after preincubation of platelets with C1q, indicating a further aspect of this tightly regulated immune reaction.[176] Interestingly, in atherogenesis, C1q-deficieny provoked a three-fold increase in

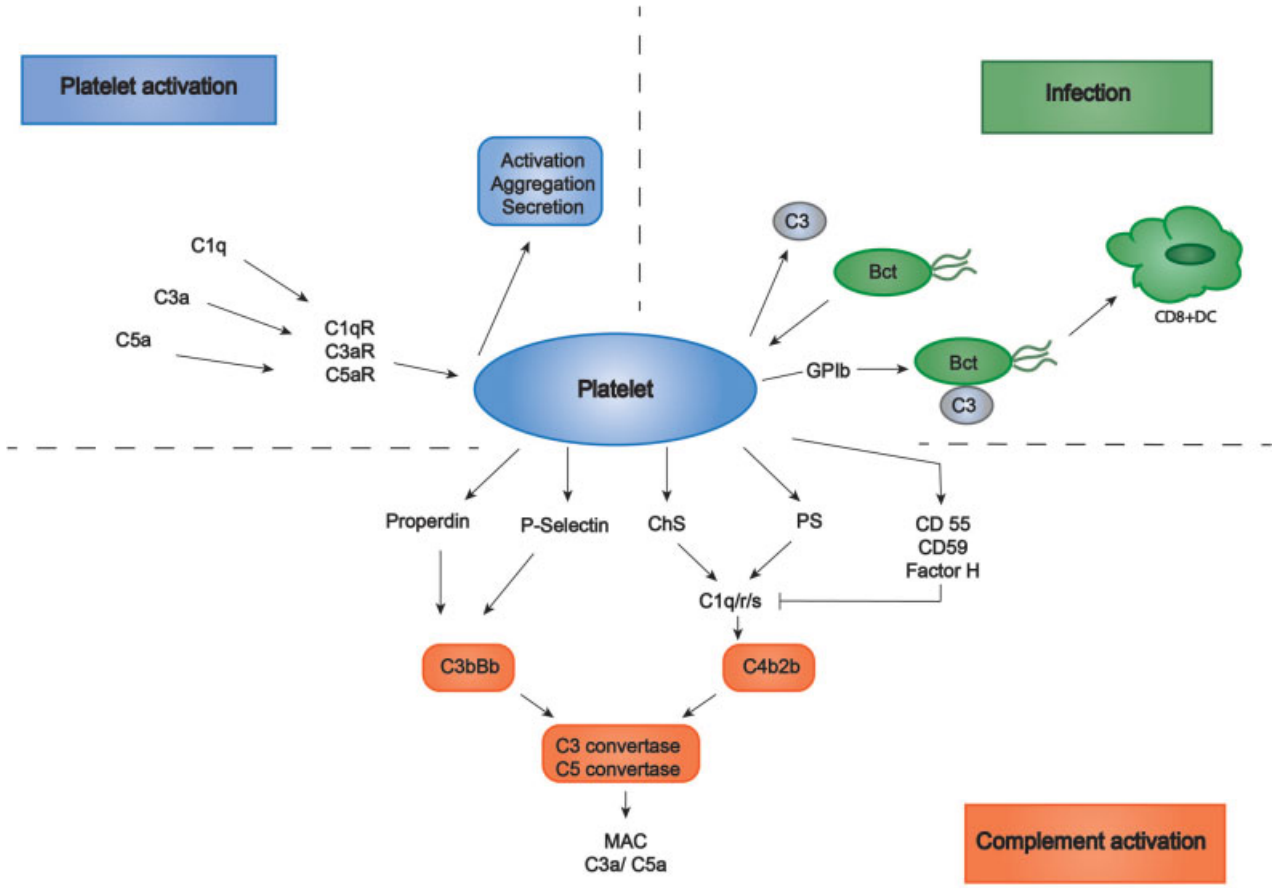

Fig. 4 Mutual stimulations of platelets and the complement cascade. Activated platelets trigger the activation of the complement system by binding complement factors on their surface. Additionally, they expose negatively charged phospholipids and release chondroitin sulfate, which both enhance complement activation. Conversely, complement factors may induce platelet activation and aggregation. Platelets protect themselves from excessive complement activation on their surface through the expression of control proteins such as CD 55 and factor H. During infection, the complement–platelet crosstalk helps direct C3-bound bacteria to immune cells in the spleen and stimulates complement release by platelets. Bct, bacteria; C3/5, complement component ⅗; C1qR, complement component C1q receptor; ChS, chondroitin sulfate; DC, dendritic cell; MAC, membrane attack complex; PS, phosphatidylserine.

lesion size compared with a control group in a model of early arteriosclerosis.[177] Other platelet complement receptors are C3aR and C5aR, which, once activated by the anaphylatoxins C3a and C5a, promote platelet aggregation, activation and serotonin release.[178–181] Surprisingly, blockade of C5a receptors had an inhibiting effect on arteriosclerotic lesion development in ApoE(−/ −) mice, whereas C3 deficiency resulted in significantly enhanced arteriosclerosis.[182,183] Recently, it was demonstrated that platelet expression of C3aR and C5aR is elevated in patients with coronary artery disease.[184] Regarding the procoagulant effects of C3a and C5a on platelets, it is now tempting to speculate that complement-mediated platelet activation might also contribute to the increase of cardiac events after viral respiratory infection. However, some aspects of platelet complement receptors remain unclear, for instance the recently discovered role of PAR¼ in the binding of C4a.[185] Furthermore, it is important to note that platelets also express multiple complement control proteins (CCP), among them are CD55, CD59 and factor H, to prevent overshooting complement activation on their surface.[186] In atypical haemolytic uremic syndrome (aHUS), one or several CCPs are mutated or deficient (mostly factor H), which leads to complement activation and MAC formation on platelets. As a result, platelets are activated and show facilitated aggregation, thereby inducing thrombophilia and microthrombosis.[187] Similarly, in paroxysmal nocturnal haemoglobinuria, deficiency in GPI anchoring of proteins on the cell membrane prevents the expression of complement regulatory proteins CD55 and CD59 on platelets, which results in complement-induced platelet activation and thrombosis.[188] Indeed, the interplay of platelets and the complement system was demonstrated to be relevant for several diseases associated with sterile inflammation. In SLE, antiphospholipid antibodies such as anti-cardiolipin antibodies, bound to platelets, activated them and enhanced deposition of C4d, a split product of C4b, on platelet surface.[189] Interestingly, C4d-deposition on platelets was associated with deep vein thrombosis, higher all-cause mortality and stroke in SLE patients.[189,190] In addition, deficiency of the VWF-cleaving protease ADAMTS13 led to complement activation and deposition on platelets bound to VWF, which probably further promoted platelet aggregation.[191] Platelet complement interactions have been shown to play a central role as well in immune defence against bacterial infections, for example via C3b-opsonized bacteria and GPIb.[28] Another recent study could prove that platelets and megakaryocytes stored C3 in their granules and that platelet activation by heat-treated *Escherichia coli*, but not LPS, triggered the translocation of C3 to the platelet surface.[192] These results suggest that platelets might contribute to immune defence through activation of the complement system after pathogen recognition. Taken together, the interactions of platelets and the complement system are essential both for sterile and infection-triggered inflammation, which makes them an interesting target for therapies in various diseases such as sepsis, arteriosclerosis, autoimmune diseases and thrombotic microangiopathy.

In conclusion, platelets show an intensive interplay with elements of the complement system, which is marked by mutual activation and regulation. Platelets have been demonstrated to facilitate the activation of both the classical and alternative complement pathway through the binding of complement components on their surface or the release of complement-inducing elements. However, activated platelets also expose complement receptors, which, upon ligation, initiate further platelet activation and aggregation. In various autoimmune diseases, dysregulation of platelet–complement interactions provokes overshooting platelet activation, and as a consequence thrombotic disorders.

Future Directions

Much progress has been made in research addressing platelet functions beyond haemostasis and there are several interesting findings which might serve as starting points for future research. In the field of ex vivo platelet production, recent stem-cell–based approaches provide an ex vivo model of megakaryopoiesis and allow the study of thrombopoiesis in the laboratory, although there are still some obstacles such as low numbers of produced platelets to overcome.[11,193] Future research might find a solution for these problems, thereby enabling the in vitro production of platelet supply for transfusions. Another rapidly growing area of interest will be the research on platelets and inflammasome in sterile inflammation. Indeed, the discovery of a platelet inflammasome and the release of HMGB1 by platelets make them a potential therapeutic target in auto-inflammatory diseases and post-ischemic injuries.[113,194] Other translational therapies might target the interaction of platelets with immune cells, such as antibodies directed against P-selectin.[195] Furthermore, inflammatory reactions contribute to platelet activation, which triggers a vicious circle of inflammation and thrombosis. Inhibitors of these interactions might serve as powerful antithrombotics without impairing haemostasis.

Concluding Remarks

The classical view of platelets as cells restricted to haemostasis has been left. Indeed, numerous studies have demonstrated how platelets tightly regulate inflammation through recruitment and activation of immune cells, release of proinflammatory factors and direct interactions with invading pathogens. However, it becomes more and more obvious that platelet functions are closely connected to each other. During inflammation, immune cells and complement factors are able to induce platelet activation and aggregation. Thus, platelets and immune cells form a complex network which enables efficient clearance of invading pathogens and death cells.

Although it is often difficult to determine the relevance of platelet–immune cell interactions to disease development and progression, the spectrum of possibly involved pathologies grows rapidly. Therefore, understanding the basic elements of this interplay is now more important than ever. The progresses made in in vitro platelet production might facilitate future research on the field of platelet interactions. Especially in sterile inflammation, therapies targeting the activation of platelets by leukocytes or elements of the

complement system and vice versa might be essential for the treatment of both cardiovascular and autoimmune diseases. However, further studies are required to clearly distinct between beneficial and pathogenic platelet mechanisms in the context of diseases.

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