

# The Mutual Relation of Platelet Activation and Innate Immunity

Felix Eisinger<sup>1</sup> Harald F. Langer<sup>1,2</sup>

<sup>1</sup>Section for Cardioimmunology, Department of Cardiovascular Medicine, University of Tuebingen, Tuebingen, Germany

<sup>2</sup>University Clinic for Cardiovascular Medicine, University of Tuebingen, Tuebingen, Germany

**Address for correspondence** Harald Langer, MD, Medizinische Klinik III, Kardiologie und Kreislaufkrankungen, Eberhard Karls-Universität Tübingen, Tübingen, Germany (e-mail: harald.langer@med.uni-tuebingen.de).

Hämostaseologie 2018;38:186–202.

## 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

- ▶ Blutplättchen
- ▶ angeborene Immunität
- ▶ Entzündung
- ▶ Infektion
- ▶ Zytokine
- ▶ Inflammasom
- ▶ Leukozyten
- ▶ Komplementsystem

received  
April 17, 2018  
accepted after revision  
July 19, 2018

© 2018 Georg Thieme Verlag KG  
Stuttgart · New York

DOI <https://doi.org/10.1055/s-0038-1669450>.  
ISSN 0720-9355.

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

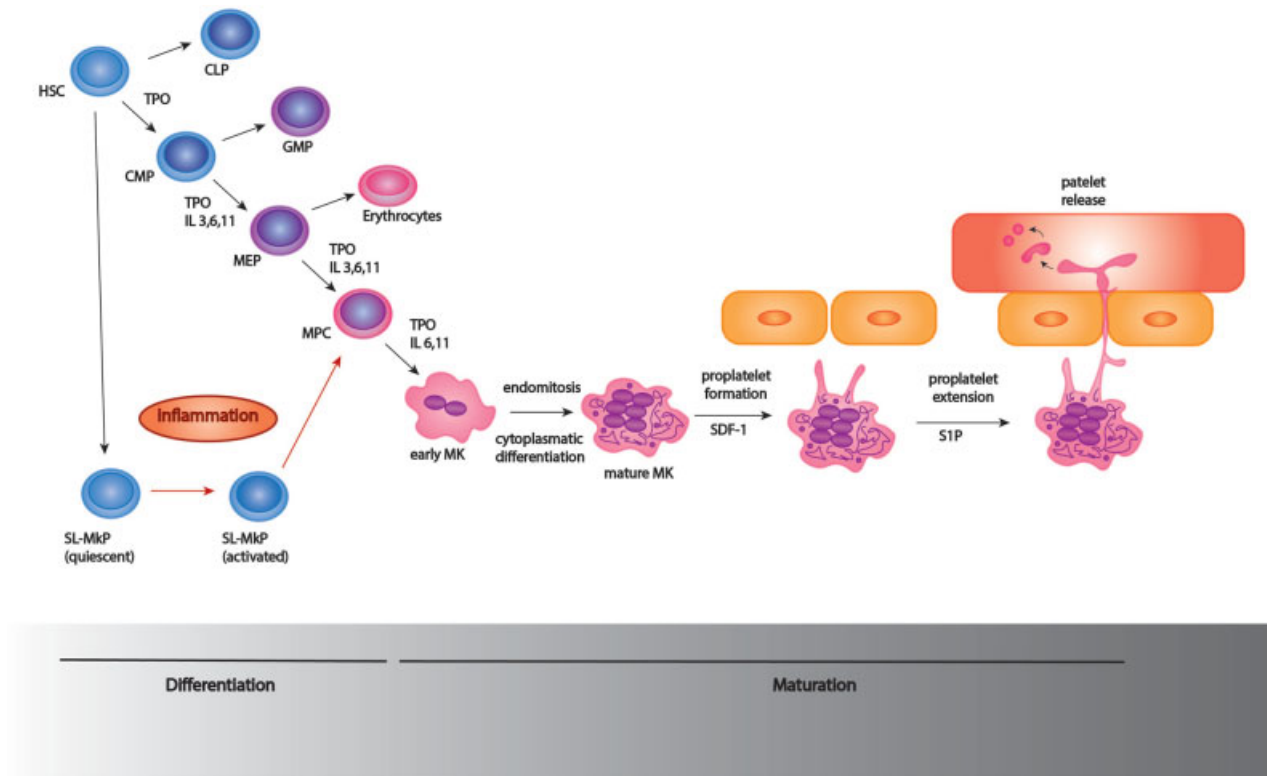
Besides leukocytes and red blood cells, platelets constitute one of the three main blood cell types. They derive from megakaryocytes, large (50–100 µm in diameter), polyploid (up to 64N) cells which are formed in the bone marrow from hematopoietic stem cells. With an average diameter of 2 to 3 µm and a concentration of 150 to 450 × 10<sup>9</sup> per litre blood, platelets play an essential role in primary haemostasis, infection, modulation of immune response and tissue remodelling.<sup>1–7</sup> 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.<sup>8</sup> 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.<sup>9–11</sup> 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.<sup>12</sup> The *classical* process of megakaryopoiesis includes several steps from multipotent progenitor cells over bipotential megakaryocytic-erythroid progenitor cells to committed megakaryocytic progenitor cells.<sup>13</sup> This development is induced by thrombopoietin (TPO) and enhanced by the cytokines interleukin- (IL-) 3, 6, 9 and 11.<sup>14–16</sup> 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).<sup>17</sup> 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.<sup>18</sup> 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.<sup>19</sup> 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<sup>+</sup>).<sup>20</sup> Another study determined that only a small subfraction of vWF<sup>+</sup>-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.<sup>21,22</sup> 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.<sup>23,24</sup> 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.<sup>13</sup> 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.<sup>25</sup>

## 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).<sup>26</sup> These observations indicate the close relationship between platelets and immune cells in inflammatory processes and any host defence against bacterial and viral pathogens.<sup>27,28</sup>

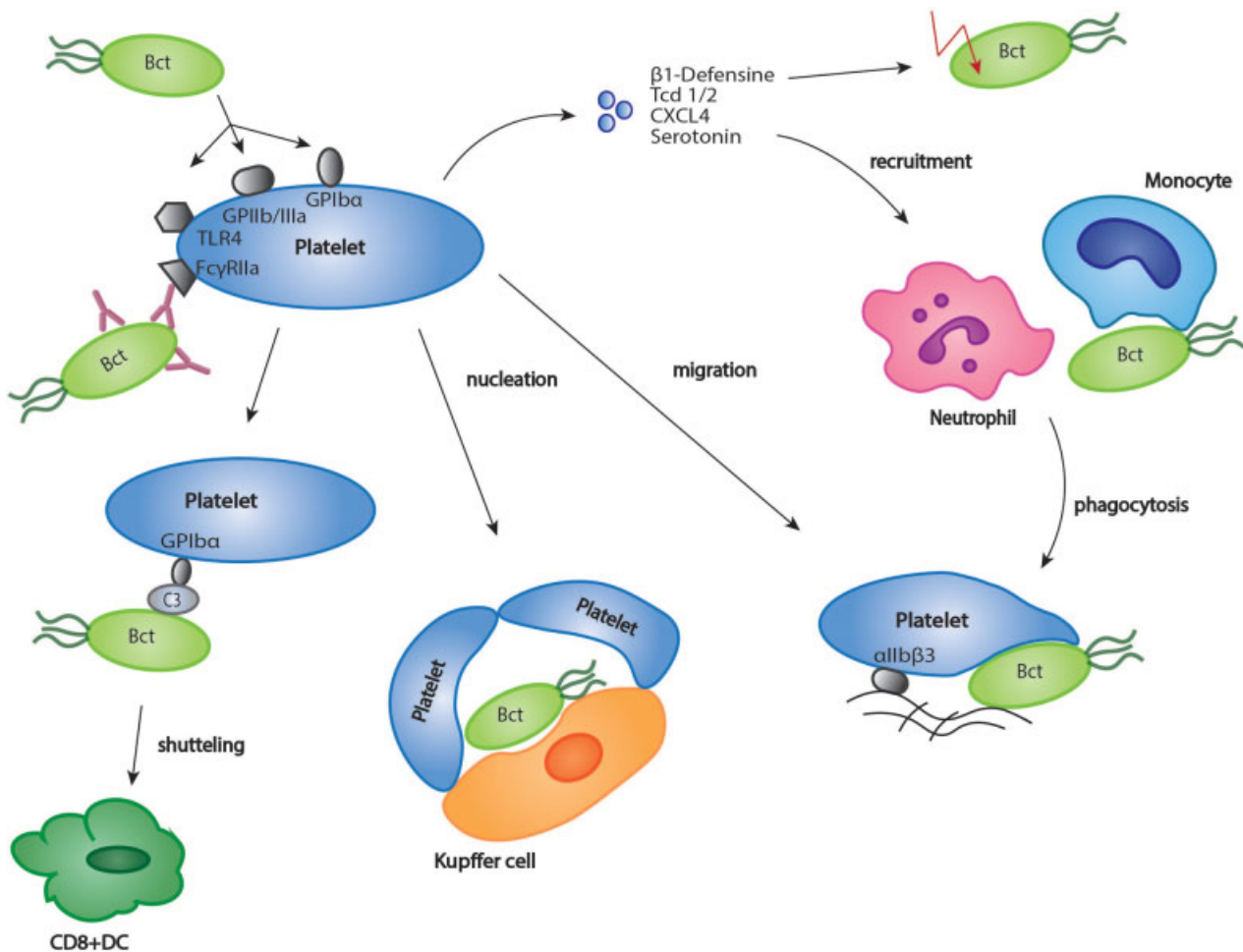
Indeed, it becomes more and more evident that platelets play an important role in bacterial and viral infections, interacting both directly with pathogens and the responding immune cells. This interplay is not only relevant for basic science but also notably contributes to clinical pathologies. A recently 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.<sup>29</sup> 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 a thrombogenic environment.<sup>29</sup> 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.<sup>30</sup> 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.<sup>31</sup> In the context of bacterial infections, platelets were shown to be activated by binding of bacterial surface antigens to platelet receptors such as GPIb $\alpha$ ,<sup>32</sup> GPIIb/IIIa<sup>33</sup> and toll-like receptor-2 (TLR2).<sup>34,35</sup> Although the specific activating ligands vary between the different bacterial strains, some studies suggested the binding of IgG-coated bacteria to platelet Fc $\gamma$ RIIA receptor as a common pathway of platelet activation, also

requiring the engagement of  $\alpha$ IIb $\beta$ 3.<sup>36,37</sup> A recently published study could prove that ATP-activated P2  $\times$  1 receptor is an essential part of this activation pathway.<sup>38</sup> 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.<sup>39</sup> In a recent study, Gaertner et al described platelet migration as a novel mechanism of bacterial clearance.<sup>40</sup> This study demonstrated that platelets are able to migrate in an actin/myosin-dependent manner 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.<sup>40</sup> Enhanced platelet-neutrophil interactions have also been reported after platelet stimulation with bacterial lipopolysaccharides (LPS).<sup>41</sup> 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.<sup>42</sup> 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; Fc $\gamma$ RIIA, Fc $\gamma$ -receptor IIA; GPIb $\alpha$ , glycoprotein Iba; GPIIb/IIIa (aIIb $\beta$ III), glycoprotein IIb/IIIa; Tcd 1/2, thrombocidin 1/2; TLR4, toll-like receptor 4.

more evident. Verschoor et al found that platelets recognized bacteria opsonized by the complement factor C3 via their GPIb $\alpha$  receptor, thereby promoting the shuttling of the complex to dendritic cells in the spleen.<sup>28</sup> This observation is in line with earlier studies that platelets can interact with antigen presenting DCs.<sup>27</sup> Indeed, activated platelets have been shown to induce the maturation of immature monocyte-derived DCs via release of CD 40 ligand.<sup>43,44</sup> Additionally, DC-induced lymphocyte proliferation was markedly enhanced in the presence of activated platelets.<sup>27</sup> Furthermore, platelets have been shown to recruit DCs through a MAC-1/JAM C and PSGL1/P-selectin dependent mechanism.<sup>45</sup> In line with this, platelets stimulated *in vivo* adhesion of DCs to an injured carotid vessel wall.<sup>27</sup> 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- $\alpha$  secretion.<sup>46</sup> 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.<sup>47</sup> Another study indicated that platelets enhance the uptake and intracellular killing of *S. aureus* by peritoneal macrophages, probably via a  $\beta$ 1-defensin-dependent mechanism,<sup>48</sup> 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,<sup>49</sup> microbicidal chemokines termed *kinocidins* such as CXCL4<sup>3,50</sup> and the defensins  $\beta$ 1 and  $\beta$ 2.<sup>39,51</sup> 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.<sup>52</sup> In line with these results, Wong et al reported an essential role of platelets in the clearance of *Bacillus cereus* infection.<sup>53</sup> 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.<sup>53</sup> 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<sup>+</sup>-T cell response, thereby promoting the development of a chronic viral hepatitis.<sup>54</sup> Furthermore, it was shown in hepatitis B virus pathogenesis that platelets adhere to liver sinusoids via CD44 and enabled the arrest of effector CD8<sup>+</sup>-T cells that cleared infected hepatocytes by extending protrusions through endothelial gaps.<sup>55</sup> 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.<sup>56</sup> 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.<sup>57</sup> Unexpectedly, it was shown that platelet-derived PF4 could be internalized by parasite-infected red blood cells via their Duffy antigen receptor for chemokines.<sup>58</sup> 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.<sup>59</sup> In reaction to these results, the use of anti-platelet drugs in clinical treatment of malaria patients was critically discussed.<sup>60</sup> However, a recent study could neither find any effect of platelet depletion on parasitaemia nor find a direct elimination of intra-erythrocytic parasites by platelets in vitro.<sup>61</sup> 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.<sup>62</sup> 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.<sup>63</sup> 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 plate-

lets showed high numbers of copies of dengue virus genome.<sup>64</sup> 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-1 $\beta$  secretion, the latter accounting for the increase in endothelial permeability.<sup>65</sup>

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.<sup>66</sup> 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.<sup>67</sup> 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.<sup>68</sup> Thus, platelets have both beneficial and detrimental effects on IE modulation. DIC is marked by extensive platelet activation and microvascular thrombosis.<sup>69</sup> 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.<sup>41,70</sup>

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

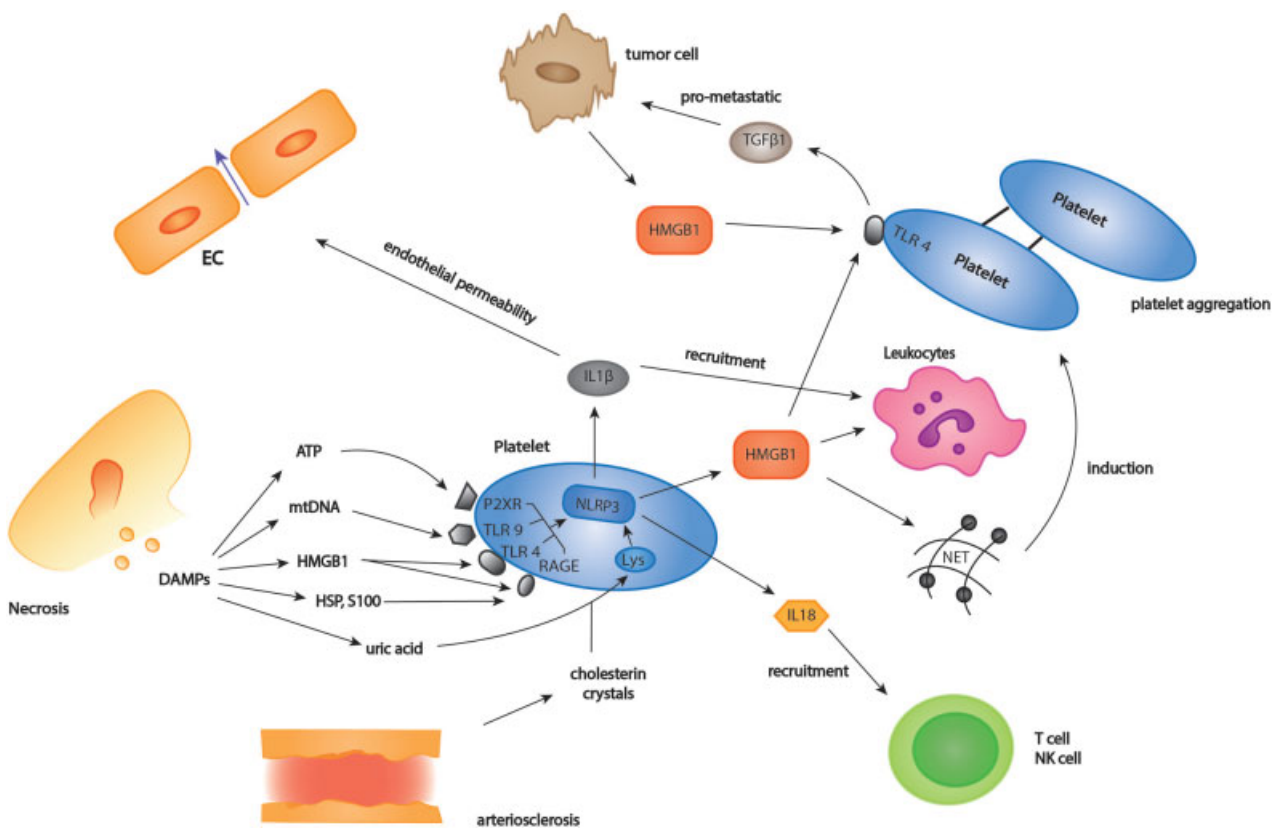
The role of platelets in inflammation has been well investigated. Platelets use a variety of receptors (CD40L, P-selectin) and cytokines (PF4, RANTES, IL1 $\beta$ ) to interact with leukocytes such as granulocytes, lymphocytes, monocytes and dendritic cells.<sup>2</sup> Indeed, one key role of platelets 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.<sup>71,72</sup> 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.<sup>73</sup> In neutrophils, platelet P-selectin could be proved to induce cell activation and release of NET.<sup>74</sup> Furthermore, the platelet-released chemokines RANTES and PF4 significantly stimulated the arrest of phagocytic cells on activated endothelium.<sup>75</sup> Surprisingly, coculture of platelets with T lymphocytes led to decreased INF $\gamma$ /TNF $\alpha$  production and reduced lymphocyte activation.<sup>76</sup>

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.<sup>77</sup> 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.<sup>78–81</sup> 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.<sup>82</sup> 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.<sup>83,84</sup> 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.<sup>85</sup> Necrotic cell ATP release has been determined to significantly induce NLRP3 inflammasome activation and subsequent IL1 $\beta$  secretion.<sup>86,87</sup> 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 inducers of sterile inflammation.<sup>88</sup> 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 HMGB1 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 1 $\beta$  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 1 $\beta$ /18, interleukin 1 $\beta$ /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; TGF $\beta$ 1, tumour growth factor  $\beta$ 1; TLR4/9, toll-like receptor 4/9.

HSP 70, can also be found among the mediators released by necrotic cells.<sup>89</sup> 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.<sup>90,91</sup> HMGB1 is a component of the cell nucleus and can be released during necrosis, but not apoptosis.<sup>92</sup> Furthermore, it promotes inflammatory reaction and elevated HMGB1 levels have been found in many inflammatory and autoimmune diseases.<sup>93,94</sup> 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.<sup>95</sup> 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.<sup>96</sup> Extracellular DNA released from the cell nucleus is present both in necrotic and apoptotic cell death.<sup>97</sup> Although bacterial DNA has been identified as a ligand of PAMP-associated TLR9 receptor, isolated endogenous DNA did not provoke an inflammatory response.<sup>98,99</sup> 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.<sup>100</sup> DAMPs may also be derived from extracellular sources, especially during extracellular matrix degradation.<sup>80</sup> 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.<sup>80</sup>

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.<sup>101-103</sup> 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.<sup>104</sup> 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.<sup>105,106</sup>

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.<sup>107-109</sup> 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.<sup>107,109</sup> Hence, CLR family shows complementary effects on sterile inflammation.

RAGE acts as a receptor for various DAMPs, among them are HMGB1, S100 and amyloid  $\beta$ .<sup>110,111</sup> Furthermore, the role of RAGE in sterile inflammation is well established. For

instance, RAGE-induced inflammatory response inhibited hepatocyte regeneration after massive liver injury.<sup>112</sup> In a model of myocardial ischemia, RAGE significantly enhanced ischemia/reperfusion injury.<sup>113</sup> 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.<sup>114</sup>

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-1 $\beta$ , IL18 and HMGB1.<sup>115,116</sup> Indeed, NLRP 3 inflammasome stimulation by cholesterol crystals has been proved to significantly induce arteriosclerotic plaque formation.<sup>117</sup> AIM2 has been originally identified as a receptor for cytosolic DNA.<sup>103</sup> 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.<sup>118</sup>

In addition to classic immune cells, platelets were shown to contribute to sterile inflammation.<sup>119</sup> Indeed, platelets express PRRs such as TLR2, 4 and 9, which are known to sense DAMPs released during tissue injury.<sup>120,121</sup> 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 TGF $\beta$ 1.<sup>122</sup> 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 NLRP3 inflammasome and consequently to the release of IL-1 $\beta$ .<sup>123</sup> Concomitantly, platelets were shown to release the proinflammatory IL18 through an inflammasome-dependent mechanism.<sup>124</sup> 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.<sup>125,126</sup> As HGMB1 has a chemoattractant and cytokine-stimulating effect on leukocytes, this indicates a possible link between platelets and immune cell regulation.<sup>127</sup> 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.<sup>128</sup> Furthermore, binding of platelet-derived HMBG1 to neutrophil RAGE was shown to trigger the release of NETs.<sup>129</sup> 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.<sup>130</sup> 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.<sup>131</sup> In line with this, platelet HMGB1 has the ability to increase thrombus formation (predominantly via TLR4).<sup>132</sup> In models of FeCl<sub>3</sub>-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.<sup>132</sup> 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.<sup>133</sup> 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.<sup>134</sup> 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.<sup>135</sup> 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.<sup>136</sup> 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.<sup>137</sup> Furthermore, malignant neoplasms are often associated with an elevated risk for thrombosis and thrombophlebitis.<sup>138</sup> In fact, cancer cells stimulate platelet activation through the release of potent mediators such as HMGB1, tissue factor, ADP and thromboxane.<sup>122,139–142</sup> Activated platelets adhere to cancer cells in the bloodstream and facilitate their arrest and migration through the vessel wall, thereby promoting tumour metastasis.<sup>143,144</sup> In addition, the activated platelets shield metastatic cancer cells from immune cell recognition and NK-mediated cell lysis.<sup>145</sup> Recently, platelets have been also shown to suppress T cell response against cancer cells via TGF- $\beta$ .<sup>146</sup> Finally, platelet granules contain

pro- and antiangiogenic factors, which are released upon tumour-dependent platelet activation.<sup>147</sup> 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  $\mu\text{m}$ , 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.<sup>148</sup> For instance, during haemostatic clot formation, PMPs significantly enhanced fibrin clot stability, whereas lack of PMPs strongly prolonged the time required for thrombin generation.<sup>149</sup> Indeed, patients with a deficiency in PMP production (Castaman's syndrome) suffer from prolonged bleeding times and haemorrhages.<sup>150</sup> Interestingly, Ponomareva et al determined that PMPs differed in size, structure and density according to the platelet-activating stimulus.<sup>151</sup> 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.<sup>152</sup> Another study demonstrated that PMPs stimulated inflammation in rheumatoid arthritis, probably via IL1-mediated activation of fibroblast-like synoviocytes.<sup>153</sup> In cancer patients, elevated PMP levels have been associated with metastasis and higher tumour aggression.<sup>154</sup> 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.<sup>155</sup> 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.<sup>156</sup> Furthermore, PMP injections significantly enhanced angiogenesis in an in vivo model of chronic myocardial ischemia.<sup>157</sup> 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.

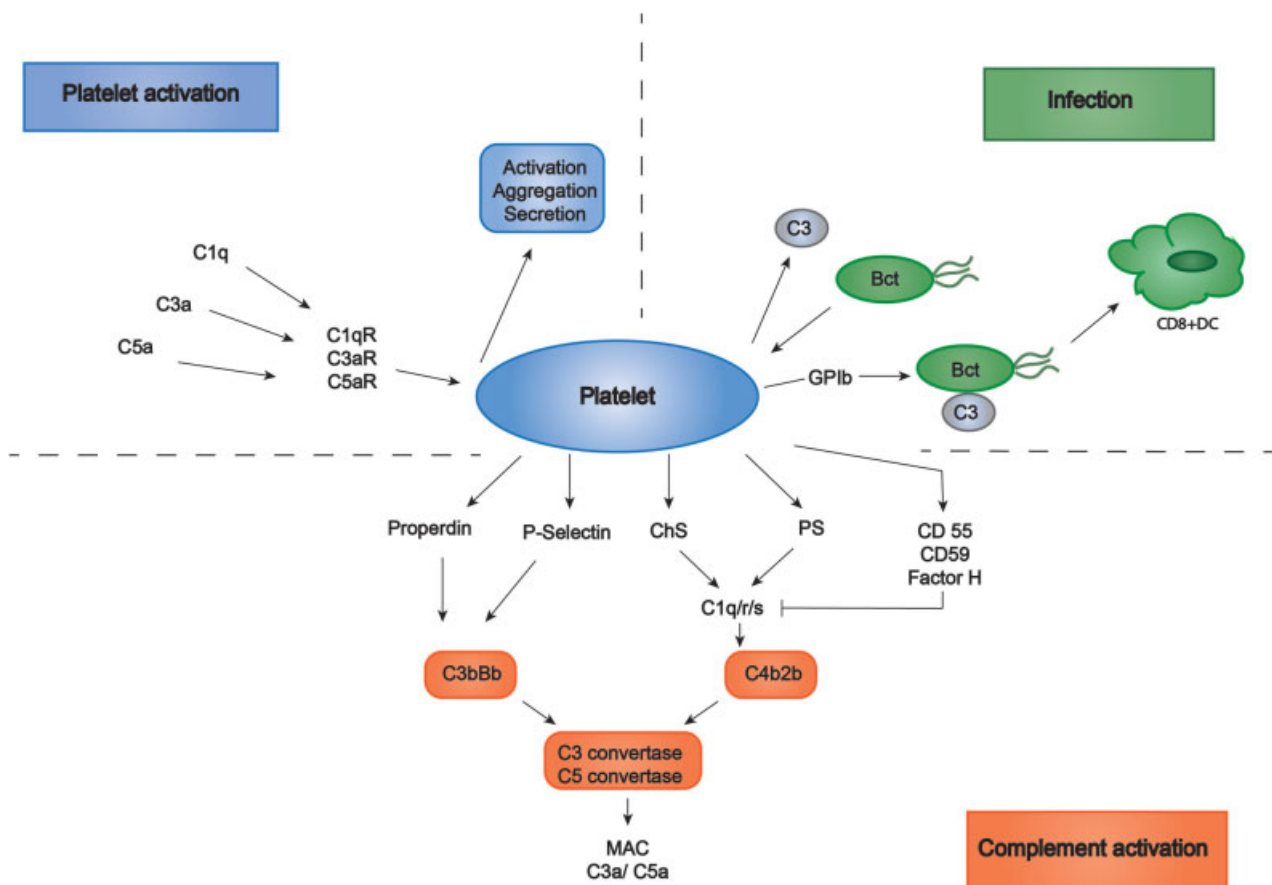
## Crosstalk Platelets—Complement

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.<sup>158,159</sup> 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).<sup>160</sup> 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.<sup>161,162</sup> Activated platelets have been shown to influence this complex network by contributing to complement activation.<sup>163</sup> 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(H<sub>2</sub>O)Bb) on their surface, which further stimulated the complement cascade.<sup>164</sup> 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.<sup>163</sup> In addition, it was shown that platelets secreted chondroitin sulfate that bound C1q and activated classical complement

pathway.<sup>165</sup> 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.<sup>166–168</sup> Yet, platelets are able to suppress complement activation via the release of C1 inhibitors.<sup>169</sup> 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.<sup>170</sup> 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.<sup>171,172</sup> The complement components C1q, C3, C4 and C9 are able to bind to the surface of activated platelets, respectively.<sup>173</sup> In line with this, platelets were shown to express C1q receptors on their surface and C1q multimers triggered platelet activation and aggregation.<sup>174,175</sup> Furthermore, platelet-neutrophil interactions were significantly decreased after preincubation of platelets with C1q, indicating a further aspect of this tightly regulated immune reaction.<sup>176</sup> Interestingly, in atherosclerosis, C1q-deficiency 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 3/5; 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.<sup>177</sup> Other platelet complement receptors are C3aR and C5aR, which, once activated by the anaphylatoxins C3a and C5a, promote platelet aggregation, activation and serotonin release.<sup>178–181</sup> Surprisingly, blockade of C5a receptors had an inhibiting effect on arteriosclerotic lesion development in ApoE(–/–) mice, whereas C3 deficiency resulted in significantly enhanced arteriosclerosis.<sup>182,183</sup> Recently, it was demonstrated that platelet expression of C3aR and C5aR is elevated in patients with coronary artery disease.<sup>184</sup> 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 PAR $\frac{1}{4}$  in the binding of C4a.<sup>185</sup> 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.<sup>186</sup> 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.<sup>187</sup> 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.<sup>188</sup> 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.<sup>189</sup> Interestingly, C4d-deposition on platelets was associated with deep vein thrombosis, higher all-cause mortality and stroke in SLE patients.<sup>189,190</sup> 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.<sup>191</sup> 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.<sup>28</sup> 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.<sup>192</sup> 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 dem-

onstrated 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.<sup>11,193</sup> 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.<sup>113,194</sup> Other translational therapies might target the interaction of platelets with immune cells, such as antibodies directed against P-selectin.<sup>195</sup> 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.

## References

- Jackson SP. Arterial thrombosis-insidious, unpredictable and deadly. *Nat Med* 2011;17(11):1423–1436
- Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* 2011;11(04):264–274
- Yeaman MR. Platelets: at the nexus of antimicrobial defence. *Nat Rev Microbiol* 2014;12(06):426–437
- Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 2013;13(01):34–45
- Li JL, Zarbock A, Hidalgo A. Platelets as autonomous drones for hemostatic and immune surveillance. *J Exp Med* 2017. Doi: 10.1084/jem.20170879. [Epub ahead of print]
- Langer HF, Choi EY, Zhou H, et al. Platelets contribute to the pathogenesis of experimental autoimmune encephalomyelitis. *Circ Res* 2012;110(09):1202–1210
- Schleicher RI, Reichenbach F, Kraft P, et al. Platelets induce apoptosis via membrane-bound FasL. *Blood* 2015;126(12):1483–1493
- Balduini A, Di Buduo CA, Kaplan DL. Translational approaches to functional platelet production ex vivo. *Thromb Haemost* 2016;115(02):250–256
- Moreau T, Evans AL, Vasquez L, et al. Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. *Nat Commun* 2016;7:11208
- Sim X, Poncz M, Gadue P, French DL. Understanding platelet generation from megakaryocytes: implications for in vitro-derived platelets. *Blood* 2016;127(10):1227–1233
- Nakamura S, Takayama N, Hirata S, et al. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell* 2014;14(04):535–548
- Wang Y, Hayes V, Jarocha D, et al. Comparative analysis of human ex vivo-generated platelets vs megakaryocyte-generated platelets in mice: a cautionary tale. *Blood* 2015;125(23):3627–3636
- Machlus KR, Italiano JE Jr. The incredible journey: from megakaryocyte development to platelet formation. *J Cell Biol* 2013;201(06):785–796
- Kaushansky K, Lok S, Holly RD, et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. *Nature* 1994;369(6481):568–571
- Ng AP, Kauppi M, Metcalf D, et al. Mpl expression on megakaryocytes and platelets is dispensable for thrombopoiesis but essential to prevent myeloproliferation. *Proc Natl Acad Sci U S A* 2014;111(16):5884–5889
- Cortin V, Garnier A, Pineault N, Lemieux R, Boyer L, Proulx C. Efficient in vitro megakaryocyte maturation using cytokine cocktails optimized by statistical experimental design. *Exp Hematol* 2005;33(10):1182–1191
- Machlus KR, Thon JN, Italiano JE Jr. Interpreting the developmental dance of the megakaryocyte: a review of the cellular and molecular processes mediating platelet formation. *Br J Haematol* 2014;165(02):227–236
- Niswander LM, Fegan KH, Kingsley PD, McGrath KE, Palis J. SDF-1 dynamically mediates megakaryocyte niche occupancy and thrombopoiesis at steady state and following radiation injury. *Blood* 2014;124(02):277–286
- Stegner D, vanEeuwijk JMM, Angay O, et al. Thrombopoiesis is spatially regulated by the bone marrow vasculature. *Nat Commun* 2017;8(01):127
- Sanjuan-Pla A, Macaulay IC, Jensen CT, et al. Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. *Nature* 2013;502(7470):232–236
- Haas S, Hansson J, Klimmeck D, et al. Inflammation-induced emergency megakaryopoiesis driven by hematopoietic stem cell-like megakaryocyte progenitors. *Cell Stem Cell* 2015;17(04):422–434
- Masamoto Y, Kurokawa M. Inflammation-induced emergency megakaryopoiesis: inflammation paves the way for platelets. *Stem Cell Investig* 2016;3:16–16
- Italiano JE, Hartwig JH. *Megakaryocyte Development and Platelet Formation*. 3rd ed. Elsevier Inc.; 2013:27–49
- Zhang L, Orban M, Lorenz M, et al. A novel role of sphingosine 1-phosphate receptor S1pr1 in mouse thrombopoiesis. *J Exp Med* 2012;209(12):2165–2181
- Lefrançois E, Ortiz-Muñoz G, Caudrillier A, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature* 2017;544(7648):105–109
- Hunt BJ. Bleeding and coagulopathies in critical care. *N Engl J Med* 2014;370(09):847–859
- Langer HF, Daub K, Braun G, et al. Platelets recruit human dendritic cells via Mac-1/JAM-C interaction and modulate dendritic cell function in vitro. *Arterioscler Thromb Vasc Biol* 2007;27(06):1463–1470
- Verschoor A, Neuenhahn M, Navarini AA, et al. A platelet-mediated system for shuttling blood-borne bacteria to CD8 $\alpha$ + dendritic cells depends on glycoprotein GPIb and complement C3. *Nat Immunol* 2011;12(12):1194–1201
- Kwong JC, Schwartz KL, Campitelli MA, et al. Acute myocardial infarction after laboratory-confirmed influenza infection. *N Engl J Med* 2018;378(04):345–353
- Kreutz RP, Bliden KP, Tantry US, Gurbel PA. Viral respiratory tract infections increase platelet reactivity and activation: an explanation for the higher rates of myocardial infarction and stroke during viral illness. *J Thromb Haemost* 2005;3(09):2108–2109
- Boilard E, Paré G, Rousseau M, et al. Influenza virus H1N1 activates platelets through Fc $\gamma$ RIIA signaling and thrombin generation. *Blood* 2014;123(18):2854–2863
- Kerrigan SW, Douglas I, Wray A, et al. A role for glycoprotein Ib in *Streptococcus sanguis*-induced platelet aggregation. *Blood* 2002;100(02):509–516
- Miajlovic H, Zapotoczna M, Geoghegan JA, Kerrigan SW, Speziale P, Foster TJ. Direct interaction of iron-regulated surface determinant IsdB of *Staphylococcus aureus* with the GPIIb/IIIa receptor on platelets. *Microbiology* 2010;156(Pt 3):920–928
- Keane C, Tilley D, Cunningham A, et al. Invasive *Streptococcus pneumoniae* trigger platelet activation via Toll-like receptor 2. *J Thromb Haemost* 2010;8(12):2757–2765
- Fitzgerald JR, Foster TJ, Cox D. The interaction of bacterial pathogens with platelets. *Nat Rev Microbiol* 2006;4(06):445–457
- Arman M, Krauel K, Tilley DO, et al. Amplification of bacteria-induced platelet activation is triggered by Fc $\gamma$ RIIA, integrin  $\alpha$ IIb $\beta$ 3, and platelet factor 4. *Blood* 2014;123(20):3166–3174
- Watson CN, Kerrigan SW, Cox D, Henderson IR, Watson SP, Arman M. Human platelet activation by *Escherichia coli*: roles for Fc $\gamma$ RIIA and integrin  $\alpha$ IIb $\beta$ 3. *Platelets* 2016;27(06):535–540
- Ilkan Z, Watson S, Watson SP, Mahaut-Smith MP. P2  $\times$  1 receptors amplify Fc $\gamma$ RIIA-induced Ca $^{2+}$  increases and functional responses in human platelets. *Thromb Haemost* 2018;118(02):369–380
- Kraemer BF, Campbell RA, Schwertz H, et al. Novel anti-bacterial activities of  $\beta$ -defensin 1 in human platelets: suppression of pathogen growth and signaling of neutrophil extracellular trap formation. *PLoS Pathog* 2011;7(11):e1002355
- Gaertner F, Ahmad Z, Rosenberger G, et al. Migrating Platelets Are Mechano-scavengers that Collect and Bundle Bacteria. *Cell* 2017;171(06):1368–1382.e23. doi: 10.1016/j.cell.2017.11.001

- 41 Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007;13(04):463–469
- 42 Duerschmied D, Suidan GL, Demers M, et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. *Blood* 2013;121(06):1008–1015
- 43 Czapiga M, Kirk AD, Lekstrom-Himes J. Platelets deliver costimulatory signals to antigen-presenting cells: a potential bridge between injury and immune activation. *Exp Hematol* 2004;32(02):135–139
- 44 Kaneider NC, Kaser A, Tilg H, Ricevuti G, Wiedermann CJ. CD40 ligand-dependent maturation of human monocyte-derived dendritic cells by activated platelets. *Int J Immunopathol Pharmacol* 2003;16(03):225–231
- 45 Maître B, Mangin PH, Eckly A, et al. Immature myeloid dendritic cells capture and remove activated platelets from preformed aggregates. *J Thromb Haemost* 2010;8(10):2262–2272
- 46 Duffau P, Seneschal J, Nicco C, et al. Platelet CD154 potentiates interferon-alpha secretion by plasmacytoid dendritic cells in systemic lupus erythematosus. *Sci Transl Med* 2010;2(47):47ra63
- 47 Krauel K, Pötschke C, Weber C, et al. Platelet factor 4 binds to bacteria, [corrected] inducing antibodies cross-reacting with the major antigen in heparin-induced thrombocytopenia. *Blood* 2011;117(04):1370–1378
- 48 Ali RA, Wuescher LM, Dona KR, Worth RG. Platelets mediate host defense against *Staphylococcus aureus* through direct bactericidal activity and by enhancing macrophage activities. *J Immunol* 2017;198(01):344–351
- 49 Krijgsveld J, Zaat SA, Meeldijk J, et al. Thrombocidins, microbicidal proteins from human blood platelets, are C-terminal deletion products of CXC chemokines. *J Biol Chem* 2000;275(27):20374–20381
- 50 Tang Y-Q, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. *Infect Immun* 2002;70(12):6524–6533
- 51 Tohidnezhad M, Varoga D, Wruck CJ, et al. Platelets display potent antimicrobial activity and release human beta-defensin 2. *Platelets* 2012;23(03):217–223
- 52 Wuescher LM, Takashima A, Worth RG. A novel conditional platelet depletion mouse model reveals the importance of platelets in protection against *Staphylococcus aureus* bacteremia. *J Thromb Haemost* 2015;13(02):303–313
- 53 Wong CH, Jenne CN, Petri B, Chrobok NL, Kubes P. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. *Nat Immunol* 2013;14(08):785–792
- 54 Lang PA, Contaldo C, Georgiev P, et al. Aggravation of viral hepatitis by platelet-derived serotonin. *Nat Med* 2008;14(07):756–761
- 55 Guidotti LG, Inverso D, Sironi L, et al. Immunosurveillance of the liver by intravascular effector CD8(+) T cells. *Cell* 2015;161(03):486–500
- 56 Iannacone M, Sitia G, Isogawa M, et al. Platelets mediate cytotoxic T lymphocyte-induced liver damage. *Nat Med* 2005;11(11):1167–1169
- 57 McMorran BJ, Marshall VM, de Graaf C, et al. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science* 2009;323(5915):797–800
- 58 McMorran BJ, Wieczorski L, Drysdale KE, et al. Platelet factor 4 and Duffy antigen required for platelet killing of *Plasmodium falciparum*. *Science* 2012;338(6112):1348–1351
- 59 Love MS, Millholland MG, Mishra S, et al. Platelet factor 4 activity against *P. falciparum* and its translation to nonpeptidic mimics as antimalarials. *Cell Host Microbe* 2012;12(06):815–823
- 60 Greenbaum DC, FitzGerald GA. Platelets, pyrexia, and plasmodia. *N Engl J Med* 2009;361(05):526–528
- 61 Gramaglia I, Velez J, Combes V, Grau GE, Wree M, van der Heyde HC. Platelets activate a pathogenic response to blood-stage *Plasmodium* infection but not a protective immune response. *Blood* 2017;129(12):1669–1679
- 62 Simmons CP, Farrar JJ, Nguyen V, Wills B. Dengue. *N Engl J Med* 2012;366(15):1423–1432
- 63 Simon AY, Sutherland MR, Prydzial EL. Dengue virus binding and replication by platelets. *Blood* 2015;126(03):378–385
- 64 Ojha A, Nandi D, Batra H, et al. Platelet activation determines the severity of thrombocytopenia in dengue infection. *Sci Rep* 2017;7:41697
- 65 Hottz ED, Lopes JF, Freitas C, et al. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammatory activation. *Blood* 2013;122(20):3405–3414
- 66 Sy RW, Chawantanpipat C, Richmond DR, Kritharides L. Thrombocytopenia and mortality in infective endocarditis. *J Am Coll Cardiol* 2008;51(18):1824–1825
- 67 Kupferwasser LI, Yeaman MR, Shapiro SM, Nast CC, Bayer AS. In vitro susceptibility to thrombin-induced platelet microbicidal protein is associated with reduced disease progression and complication rates in experimental *Staphylococcus aureus* endocarditis: microbiological, histopathologic, and echocardiographic analyses. *Circulation* 2002;105(06):746–752
- 68 Jung C-J, Yeh C-Y, Shun C-T, et al. Platelets enhance biofilm formation and resistance of endocarditis-inducing streptococci on the injured heart valve. *J Infect Dis* 2012;205(07):1066–1075
- 69 Levi M, Ten Cate H. Disseminated intravascular coagulation. *N Engl J Med* 1999;341(08):586–592
- 70 McDonald B, Davis RP, Kim S-J, et al. Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood* 2017;129(10):1357–1367
- 71 Palabrica T, Lobb R, Furie BC, et al. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. *Nature* 1992;359(6398):848–851
- 72 Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. *Blood* 1996;88(01):146–157
- 73 Passacuale G, Vamadevan P, Pereira L, Hamid C, Corrigan V, Ferro A. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. *PLoS One* 2011;6(10):e25595
- 74 Etulain J, Martinod K, Wong SL, Cifuni SM, Schattner M, Wagner DD. P-selectin promotes neutrophil extracellular trap formation in mice. *Blood* 2015;126(02):242–246
- 75 von Hundelshausen P, Koenen RR, Sack M, et al. Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. *Blood* 2005;105(03):924–930
- 76 Zamora C, Cantó E, Nieto JC, et al. Binding of platelets to lymphocytes: a potential anti-inflammatory therapy in rheumatoid arthritis. *J Immunol* 2017;198(08):3099–3108
- 77 Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol* 2010;10(12):826–837
- 78 Eltzschig HK, Eckle T. Ischemia and reperfusion—from mechanism to translation. *Nat Med* 2011;17(11):1391–1401
- 79 Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest* 2005;115(12):3378–3384
- 80 Jiang D, Liang J, Fan J, et al. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 2005;11(11):1173–1179
- 81 Shen H, Kreisel D, Goldstein DR. Processes of sterile inflammation. *J Immunol* 2013;191(06):2857–2863
- 82 Schaefer L. Complexity of danger: the diverse nature of damage-associated molecular patterns. *J Biol Chem* 2014;289(51):35237–35245
- 83 Chekeni FB, Elliott MR, Sandilos JK, et al. Pannexin 1 channels mediate ‘find-me’ signal release and membrane permeability during apoptosis. *Nature* 2010;467(7317):863–867

- 84 Elliott MR, Chekeni FB, Trampont PC, et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* 2009;461(7261):282–286
- 85 Sáez PJ, Vargas P, Shoji KF, Harcha PA, Lennon-Duménil A-M, Sáez JC. ATP promotes the fast migration of dendritic cells through the activity of pannexin 1 channels and P2X<sub>7</sub> receptors. *Sci Signal* 2017;10(506):7107
- 86 Iyer SS, Pulskens WP, Sadler JJ, et al. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc Natl Acad Sci U S A* 2009;106(48):20388–20393
- 87 Cauwels A, Rogge E, Vandendriessche B, Shiva S, Brouckaert P. Extracellular ATP drives systemic inflammation, tissue damage and mortality. *Cell Death Dis* 2014;5(03):e1102
- 88 Kono H, Chen C-J, Ontiveros F, Rock KL. Uric acid promotes an acute inflammatory response to sterile cell death in mice. *J Clin Invest* 2010;120(06):1939–1949
- 89 Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF- $\kappa$  B pathway. *Int Immunol* 2000;12(11):1539–1546
- 90 Millar DG, Garza KM, Odermatt B, et al. Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo. *Nat Med* 2003;9(12):1469–1476
- 91 Fang H, Wu Y, Huang X, et al. Toll-like receptor 4 (TLR4) is essential for Hsp70-like protein 1 (HSP70L1) to activate dendritic cells and induce Th1 response. *J Biol Chem* 2011;286(35):30393–30400
- 92 Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418(6894):191–195
- 93 Magna M, Pisetsky DS. The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. *Mol Med* 2014;20(01):138–146
- 94 Rovere-Querini P, Capobianco A, Scaffidi P, et al. HMGB1 is an endogenous immune adjuvant released by necrotic cells. *EMBO Rep* 2004;5(08):825–830
- 95 Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT. Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. *J Immunol* 2011;187(05):2626–2631
- 96 Kawai C, Kotani H, Miyao M, et al. Circulating extracellular histones are clinically relevant mediators of multiple organ injury. *Am J Pathol* 2016;186(04):829–843
- 97 Tran TT, Groben P, Pisetsky DS. The release of DNA into the plasma of mice following hepatic cell death by apoptosis and necrosis. *Biomarkers* 2008;13(02):184–200
- 98 Ohto U, Shibata T, Tanji H, et al. Structural basis of CpG and inhibitory DNA recognition by Toll-like receptor 9. *Nature* 2015;520(7549):702–705
- 99 Pisetsky DS. The origin and properties of extracellular DNA: from PAMP to DAMP. *Clin Immunol* 2012;144(01):32–40
- 100 Urbonaviciute V, Fürtrohr BG, Meister S, et al. Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE. *J Exp Med* 2008;205(13):3007–3018
- 101 Piccinini AM, Midwood KS. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm* 2010;2010:xx
- 102 Fritz JH, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. *Nat Immunol* 2006;7(12):1250–1257
- 103 Hornung V, Ablasser A, Charrel-Dennis M, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 2009;458(7237):514–518
- 104 Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol* 2014;5:461
- 105 He C, Lai P, Wang J, et al. TLR2/4 deficiency prevents oxygen-induced vascular degeneration and promotes revascularization by downregulating IL-17 in the retina. *Sci Rep* 2016;6(01):27739
- 106 Mayer C, Adam M, Glashauser L, et al. Sterile inflammation as a factor in human male infertility: involvement of Toll like receptor 2, biglycan and peritubular cells. *Sci Rep* 2016;6(01):37128
- 107 Neumann K, Castiñeiras-Vilariño M, Höckendorf U, et al. Clec12a is an inhibitory receptor for uric acid crystals that regulates inflammation in response to cell death. *Immunity* 2014;40(03):389–399
- 108 Ahrens S, Zelenay S, Sancho D, et al. F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells. *Immunity* 2012;36(04):635–645
- 109 Yamasaki S, Ishikawa E, Sakuma M, Hara H, Ogata K, Saito T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat Immunol* 2008;9(10):1179–1188
- 110 Kokkola R, Andersson A, Mullins G, et al. RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. *Scand J Immunol* 2005;61(01):1–9
- 111 Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. *Annu Rev Immunol* 2010;28(01):367–388
- 112 Cataldegirmen G, Zeng S, Feirt N, et al. RAGE limits regeneration after massive liver injury by coordinated suppression of TNF- $\alpha$  and NF- $\kappa$ B. *J Exp Med* 2005;201(03):473–484
- 113 Bucciarelli LG, Kaneko M, Ananthakrishnan R, et al. Receptor for advanced-glycation end products: key modulator of myocardial ischemic injury. *Circulation* 2006;113(09):1226–1234
- 114 Bangert A, Andrassy M, Müller A-M, et al. Critical role of RAGE and HMGB1 in inflammatory heart disease. *Proc Natl Acad Sci U S A* 2016;113(02):E155–E164
- 115 Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- $\beta$ . *Mol Cell* 2002;10(02):417–426
- 116 Willingham SB, Allen IC, Bergstralh DT, et al. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol* 2009;183(03):2008–2015
- 117 Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010;464(7293):1357–1361
- 118 Denes A, Coutts G, Lénárt N, et al. AIM2 and NLR4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3. *Proc Natl Acad Sci U S A* 2015;112(13):4050–4055
- 119 Vogel S, Thein SL. Platelets at the crossroads of thrombosis, inflammation and haemolysis. *Br J Haematol* 2018;180(05):761–767
- 120 Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of Toll-like receptor molecules on human platelets. *Immunol Cell Biol* 2005;83(02):196–198
- 121 Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional Toll-like receptor-4. *Blood* 2005;106(07):2417–2423
- 122 Yu L-X, Yan L, Yang W, et al. Platelets promote tumour metastasis via interaction between TLR4 and tumour cell-released high-mobility group box1 protein. *Nat Commun* 2014;5:5256
- 123 Chi W, Chen H, Li F, Zhu Y, Yin W, Zhuo Y. HMGB1 promotes the activation of NLRP3 and caspase-8 inflammasomes via NF- $\kappa$ B pathway in acute glaucoma. *J Neuroinflammation* 2015;12(01):137
- 124 Allam O, Samarani S, Jenabian M-A, et al. Differential synthesis and release of IL-18 and IL-18 Binding Protein from human platelets and their implications for HIV infection. *Cytokine* 2017;90:144–154
- 125 Maugeri N, Franchini S, Campana L, et al. Circulating platelets as a source of the damage-associated molecular pattern HMGB1 in patients with systemic sclerosis. *Autoimmunity* 2012;45(08):584–587
- 126 Rouhiainen A, Imai S, Rauvala H, Parkkinen J. Occurrence of amphoterin (HMG1) as an endogenous protein of human

- platelets that is exported to the cell surface upon platelet activation. *Thromb Haemost* 2000;84(06):1087–1094
- 127 Venereau E, Casalgrandi M, Schiraldi M, et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *J Exp Med* 2012;209(09):1519–1528
  - 128 Vogel S, Rath D, Borst O, et al. Platelet-derived high-mobility group box 1 promotes recruitment and suppresses apoptosis of monocytes. *Biochem Biophys Res Commun* 2016;478(01):143–148
  - 129 Maugeri N, Campana L, Gavina M, et al. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. *J Thromb Haemost* 2014;12(12):2074–2088
  - 130 Yang X, Wang H, Zhang M, Liu J, Lv B, Chen F. HMGB1: a novel protein that induced platelets active and aggregation via Toll-like receptor-4, NF- $\kappa$ B and cGMP dependent mechanisms. *Diagn Pathol* 2015;10(01):134
  - 131 Ahrens I, Chen Y-C, Topcic D, et al. HMGB1 binds to activated platelets via the receptor for advanced glycation end products and is present in platelet rich human coronary artery thrombi. *Thromb Haemost* 2015;114(05):994–1003
  - 132 Vogel S, Bodenstern R, Chen Q, et al. Platelet-derived HMGB1 is a critical mediator of thrombosis. *J Clin Invest* 2015;125(12):4638–4654
  - 133 Stark K, Philippi V, Stockhausen S, et al. Disulfide HMGB1 derived from platelets coordinates venous thrombosis in mice. *Blood* 2016;128(20):2435–2449
  - 134 Murthy P, Durco F, Miller-Ocuin JL, et al. The NLRP3 inflammatory and bruton's tyrosine kinase in platelets co-regulate platelet activation, aggregation, and in vitro thrombus formation. *Biochem Biophys Res Commun* 2017;483(01):230–236
  - 135 Dyer MR, Chen Q, Haldeman S, et al. Deep vein thrombosis in mice is regulated by platelet HMGB1 through release of neutrophil-extracellular traps and DNA. *Sci Rep* 2018;8(01):2068
  - 136 Mardente S, Mari E, Massimi I, et al. From human megakaryocytes to platelets: effects of aspirin on high-mobility group box 1/receptor for advanced glycation end products axis. *Front Immunol* 2018;8:1946
  - 137 Bailey SE, Ukoumunne OC, Shephard EA, Hamilton W. Clinical relevance of thrombocytosis in primary care: a prospective cohort study of cancer incidence using English electronic medical records and cancer registry data. *Br J Gen Pract* 2017;67(659):e405–e413
  - 138 Jain S, Harris J, Ware J. Platelets: linking hemostasis and cancer. *Arterioscler Thromb Vasc Biol* 2010;30(12):2362–2367
  - 139 Khorana AA, Ahrendt SA, Ryan CK, et al. Tissue factor expression, angiogenesis, and thrombosis in pancreatic cancer. *Clin Cancer Res* 2007;13(10):2870–2875
  - 140 Cho MS, Noh K, Haemmerle M, et al. Role of ADP receptors on platelets in the growth of ovarian cancer. *Blood* 2017;130(10):1235–1242
  - 141 Haemmerle M, Bottsford-Miller J, Pradeep S, et al. FAK regulates platelet extravasation and tumor growth after antiangiogenic therapy withdrawal. *J Clin Invest* 2016;126(05):1885–1896
  - 142 Sakai H, Suzuki T, Takahashi Y, et al. Upregulation of thromboxane synthase in human colorectal carcinoma and the cancer cell proliferation by thromboxane A<sub>2</sub>. *FEBS Lett* 2006;580(14):3368–3374
  - 143 Karpatkin S, Pearlstein E, Ambrogio C, Collier BS. Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo. *J Clin Invest* 1988;81(04):1012–1019
  - 144 Weber MR, Zuka M, Lorger M, et al. Activated tumor cell integrin  $\alpha$ v $\beta$ 3 cooperates with platelets to promote extravasation and metastasis from the blood stream. *Thromb Res* 2016;140(Suppl 1):S27–S36
  - 145 Palumbo JS, Talmage KE, Massari JV, et al. Platelets and fibrin (ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood* 2005;105(01):178–185
  - 146 Rachidi S, Metelli A, Riesenberger B, et al. Platelets subvert T cell immunity against cancer via GARP-TGF $\beta$  axis. *Sci Immunol* 2017;2(11):7911
  - 147 Battinelli EM, Markens BA, Italiano JE Jr. Release of angiogenesis regulatory proteins from platelet alpha granules: modulation of physiologic and pathologic angiogenesis. *Blood* 2011;118(05):1359–1369
  - 148 Varon D, Shai E. Platelets and their microparticles as key players in pathophysiological responses. *J Thromb Haemost* 2015;13(Suppl 1):S40–S46
  - 149 Zubairova LD, Nabiullina RM, Nagaswami C, et al. Circulating microparticles alter formation, structure, and properties of fibrin clots. *Sci Rep* 2015;5(01):17611
  - 150 Castaman G, Yu-Feng L, Rodeghiero F. A bleeding disorder characterised by isolated deficiency of platelet microvesicle generation. *Lancet* 1996;347(9002):700–701
  - 151 Ponomareva AA, Nevzorova TA, Mordakhanova ER, et al. Intracellular origin and ultrastructure of platelet-derived microparticles. *J Thromb Haemost* 2017;15(08):1655–1667
  - 152 Boudreau LH, Duchez A-C, Cloutier N, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A<sub>2</sub> to promote inflammation. *Blood* 2014;124(14):2173–2183
  - 153 Boilard E, Nigrovic PA, Larabee K, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* 2010;327(5965):580–583
  - 154 Kim HK, Song KS, Park YS, et al. Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. *Eur J Cancer* 2003;39(02):184–191
  - 155 Michael JV, Wurtzel JGT, Mao GF, et al. Platelet microparticles infiltrating solid tumors transfer miRNAs that suppress tumor growth. *Blood* 2017;130(05):567–580
  - 156 Kim HK, Song KS, Chung J-H, Lee KR, Lee S-N. Platelet microparticles induce angiogenesis in vitro. *Br J Haematol* 2004;124(03):376–384
  - 157 Brill A, Dashevsky O, Rivo J, Gozal Y, Varon D. Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. *Cardiovasc Res* 2005;67(01):30–38
  - 158 Sarma JV, Ward PA. The complement system. *Cell Tissue Res* 2011;343(01):227–235
  - 159 Patzelt J, Verschoor A, Langer HF. Platelets and the complement cascade in atherosclerosis. *Front Physiol* 2015;6:49
  - 160 Mathern DR, Heeger PS. Molecules great and small: the complement system. *Clin J Am Soc Nephrol* 2015;10(09):1636–1650
  - 161 Nording H, Langer HF. Complement links platelets to innate immunity. *Semin Immunol* 2018;37:43–52
  - 162 Carroll MC. The complement system in regulation of adaptive immunity. *Nat Immunol* 2004;5(10):981–986
  - 163 Del Conde I, Cruz MA, Zhang H, López JA, Afshar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. *J Exp Med* 2005;201(06):871–879
  - 164 Saggi G, Cortes C, Emch HN, Ramirez G, Worth RG, Ferreira VP. Identification of a novel mode of complement activation on stimulated platelets mediated by properdin and C3(H<sub>2</sub>O). *J Immunol* 2013;190(12):6457–6467
  - 165 Hamad OA, Ekdahl KN, Nilsson PH, et al. Complement activation triggered by chondroitin sulfate released by thrombin receptor-activated platelets. *J Thromb Haemost* 2008;8(08):1413–1421
  - 166 Bevers EM, Comfurius P, Zwaal RF. Changes in membrane phospholipid distribution during platelet activation. *Biochim Biophys Acta* 1983;736(01):57–66
  - 167 Kovacovics T, Tschopp J, Kress A, Isliker H. Antibody-independent activation of C1, the first component of complement, by cardiolipin. *J Immunol* 1985;135(04):2695–2700
  - 168 Païdassi H, Tacnet-Delorme P, Garlatti V, et al. C1q binds phosphatidylserine and likely acts as a multiligand-bridging

- molecule in apoptotic cell recognition. *J Immunol* 2008;180(04):2329–2338
- 169 Schmaier AH, Amenta S, Xiong T, Heda GD, Gewirtz AM. Expression of platelet C1 inhibitor. *Blood* 1993;82(02):465–474
- 170 Shanmugavelayudam SK, Rubenstein DA, Yin W. Effects of physiologically relevant dynamic shear stress on platelet complement activation. *Platelets* 2011;22(08):602–610
- 171 Gushiken FC, Han H, Li J, Rumbaut RE, Afshar-Kharghan V. Abnormal platelet function in C3-deficient mice. *J Thromb Haemost* 2009;7(05):865–870
- 172 Subramaniam S, Jurk K, Hobohm L, et al. Distinct contributions of complement factors to platelet activation and fibrin formation in venous thrombus development. *Blood* 2017;129(16):2291–2302
- 173 Hamad OA, Nilsson PH, Wouters D, Lambris JD, Ekdahl KN, Nilsson B. Complement component C3 binds to activated normal platelets without preceding proteolytic activation and promotes binding to complement receptor 1. *J Immunol* 2010;184(05):2686–2692
- 174 Peerschke EI, Reid KB, Ghebrehiwet B. Platelet activation by C1q results in the induction of alpha IIb/beta 3 integrins (GPIIb-IIIa) and the expression of P-selectin and procoagulant activity. *J Exp Med* 1993;178(02):579–587
- 175 Peerschke EI, Ghebrehiwet B. Platelet receptors for the complement component C1q: implications for hemostasis and thrombosis. *Immunobiology* 1998;199(02):239–249
- 176 Skoglund C, Wetterö J, Tengvall P, Bengtsson T. C1q induces a rapid up-regulation of P-selectin and modulates collagen- and collagen-related peptide-triggered activation in human platelets. *Immunobiology* 2010;215(12):987–995
- 177 Bhatia VK, Yun S, Leung V, et al. Complement C1q reduces early atherosclerosis in low-density lipoprotein receptor-deficient mice. *Am J Pathol* 2007;170(01):416–426
- 178 Fukuoka Y, Hugli TE. Demonstration of a specific C3a receptor on guinea pig platelets. *J Immunol* 1988;140(10):3496–3501
- 179 Polley MJ, Nachman RL. Human platelet activation by C3a and C3a des-arg. *J Exp Med* 1983;158(02):603–615
- 180 Kretzschmar T, Kahl K, Rech K, Bautsch W, Köhl J, Bitter-Suermann D. Characterization of the C5a receptor on guinea pig platelets. *Immunobiology* 1991;183(05):418–432
- 181 Meuer S, Ecker U, Hadding U, Bitter-Suermann D. Platelet-serotonin release by C3a and C5a: two independent pathways of activation. *J Immunol* 1981;126(04):1506–1509
- 182 Persson L, Borén J, Robertson A-KL, Wallenius V, Hansson GK, Pekna M. Lack of complement factor C3, but not factor B, increases hyperlipidemia and atherosclerosis in apolipoprotein E-/- low-density lipoprotein receptor-/- mice. *Arterioscler Thromb Vasc Biol* 2004;24(06):1062–1067
- 183 Manthey HD, Thomas AC, Shiels IA, et al. Complement C5a inhibition reduces atherosclerosis in ApoE-/- mice. *FASEB J* 2011;25(07):2447–2455
- 184 Patzelt J, Mueller KA, Breuning S, et al. Expression of anaphylatoxin receptors on platelets in patients with coronary heart disease. *Atherosclerosis* 2015;238(02):289–295
- 185 Wang H, Ricklin D, Lambris JD. Complement-activation fragment C4a mediates effector functions by binding as untethered agonist to protease-activated receptors 1 and 4. *Proc Natl Acad Sci U S A* 2017;114(41):10948–10953
- 186 Verschoor A, Langer HF. Crosstalk between platelets and the complement system in immune protection and disease. *Thromb Haemost* 2013;110(05):910–919
- 187 Jokiranta TS. HUS and atypical HUS. *Blood* 2017;129(21):2847–2856
- 188 Hill A, Kelly RJ, Hillmen P. Thrombosis in paroxysmal nocturnal hemoglobinuria. *Blood* 2013;121(25):4985–4996, quiz 5105
- 189 Lood C, Tydén H, Gullstrand B, et al. Platelet activation and anti-phospholipid antibodies collaborate in the activation of the complement system on platelets in systemic lupus erythematosus. *PLoS One* 2014;9(06):e99386
- 190 Kao AH, McBurney CA, Sattar A, et al. Relation of platelet C4d with all-cause mortality and ischemic stroke in patients with systemic lupus erythematosus. *Transl Stroke Res* 2014;5(04):510–518
- 191 Tati R, Kristoffersson A-C, Ståhl A-L, et al. Complement activation associated with ADAMTS13 deficiency in human and murine thrombotic microangiopathy. *J Immunol* 2013;191(05):2184–2193
- 192 Arbesu I, Bucsaiova M, Fischer MB, Mannhalter C. Platelet-borne complement proteins and their role in platelet-bacteria interactions. *J Thromb Haemost* 2016;14(11):2241–2252
- 193 Pietrzyk-Nivau A, Poirault-Chassac S, Gandrille S, et al. Three-dimensional environment sustains hematopoietic stem cell differentiation into platelet-producing megakaryocytes. *PLoS One* 2015;10(08):e0136652
- 194 Lundbäck P, Klevenvall L, Ottosson L, et al. Anti HMGB1 treatment reduces inflammation in models of experimental autoimmunity. *Ann Rheum Dis* 2012;71(Suppl 1):A79–A80
- 195 Stähli BE, Tardif J-C, Carrier M, et al. Effects of P-selectin antagonist inclacumab in patients undergoing coronary artery bypass graft surgery: SELECT-CABG trial. *J Am Coll Cardiol* 2016;67(03):344–346