The Concept of Thromboinflammation

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Abstract

Keywords
► thromboinflammation
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► leukocytes
► complement

Inflammation and thrombosis are intricate and closely interconnected biological processes that are not yet fully understood and lack effective targeted therapeutic approaches. Thrombosis initiated by inflammatory responses, known as immunothrombosis, can confer advantages to the host by constraining the spread of pathogens within the bloodstream. Conversely, platelets and the coagulation cascade can influence inflammatory responses through interactions with immune cells, endothelium, or complement system. These interactions can lead to a state of heightened inflammation resulting from thrombotic processes, termed as thromboinflammation. This review aims to comprehensively summarize the existing knowledge of thromboinflammation and addressing its significance as a challenging clinical issue.

Zusammenfassung

Schlüsselwörter
► Thromboinflammation
► Immunthrombose
► Blutplättchen
► Gerinnung
► Leukozyten
► Komplement

Entzündungen und Thrombosen sind komplizierte und eng miteinander verwobene biologische Prozesse, die noch nicht vollständig aufgeklärt sind und denen es an wirksamen gezielten Therapieansätzen mangelt. Eine durch Entzündungsreaktionen ausgelöste Thrombose, bekannt als Immunthrombose, kann Vorteile bringen, indem sie die Ausbreitung von Krankheitserregern im Blutkreislauf einschränkt. Umgekehrt können Thrombozyten und die Gerinnungskaskade durch Interaktionen mit Immunzellen, dem Endothel oder dem Komplementsystem Entzündungsreaktionen beeinflussen. Diese Wechselwirkungen können zu einem Zustand verstärkter Entzündung führen, der aus thrombotischen Prozessen resultiert und daher als Thromboinflammation bezeichnet wird. Dieser Übersichtsartikel zielt darauf ab, das vorhandene Wissen über Thromboinflammation umfassend zusammenzufassen und sich mit ihrer Bedeutung als anspruchsvolles klinisches Problem zu befassen, das noch nicht ausreichend verstanden und auch nicht therapeutisch behandelt wird.

From Immunothrombosis to Thromboinflammation

The term immunothrombosis was first coined in 2013 in a review by Engelmann and Massberg, to describe the mechanisms by which the innate immune system can trigger thrombotic events.[1] Immunothrombosis involves an interplay of innate immune cells, platelets, and coagulation factors, which contributes to the local control and clearance of infections.[2] In its physiological form, it represents a process of microcoagulation, which does not lead to adverse clinical symptoms and simply helps to immobilize invading pathogens or foreign "danger" structures for subsequent clearance by immune cells. However, excessive inflammatory reactions can also induce coagulopathies and harmful thrombotic events, emphasizing a fine-balanced interplay between the immune and the hemostatic systems (►Fig. 1).
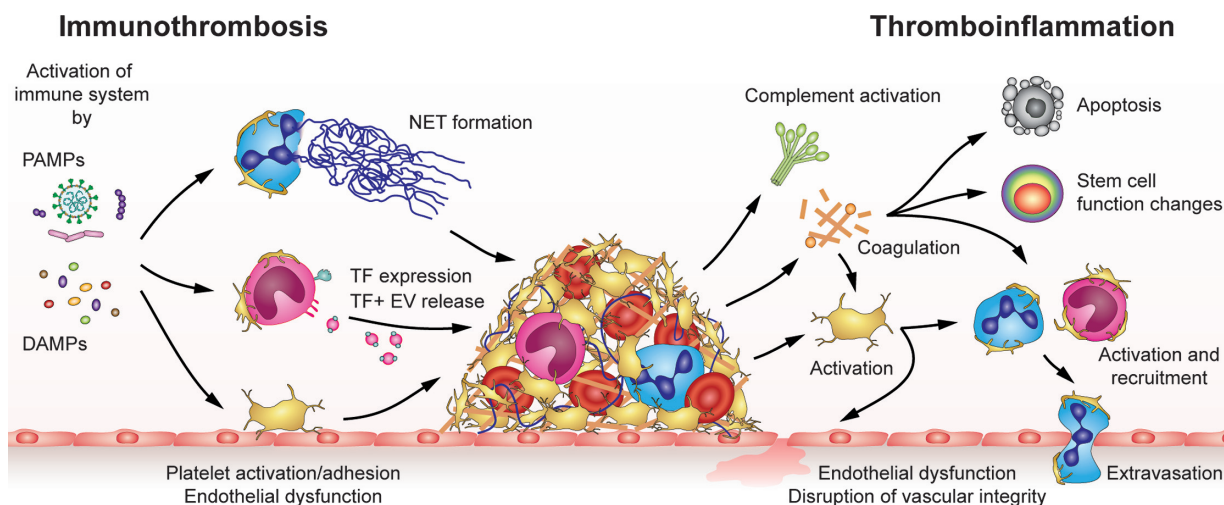
Fig. 1 From immunothrombosis to thromboinflammation. Activation of the immune system by PAMPs (e.g., in viral/bacterial infection) or DAMPs (e.g., in hemolytic disease, ischemia/reperfusion injury) triggers procoagulant NET formation and monocyte TF expression and release of TF + EVs. Activated platelets act as threshold switch and critically enhance these processes, which in turn foster thrombin formation and further platelet activation. Concomitant endothelial dysfunction results in loss of anticoagulant mechanisms and platelet adhesion, leading to the development of intravascular thrombosis. Thrombotic processes in turn trigger the dysregulated activation of complement, coagulation, platelets, and endothelial cells, which leads to disrupted vascular integrity. Aberrant activation of coagulation factors and platelets mediates inflammation by augmenting recruitment and extravasation of immune cells, particularly of neutrophils and monocytes. In addition, dysregulated induction of coagulation may influence immune cell turnover by impacting on both apoptosis and stem cell functions. DAMP, danger-associated molecular pattern, EV, extracellular vesicle, NET, neutrophil extracellular trap, PAMP, pathogen-associated molecular pattern, TF, tissue factor.

The term thromboinflammation was already used prior to immunothrombosis to describe various aspects of platelet involvement in inflammatory processes. In 2004, it was first used to describe platelet–leukocyte interactions,[3] whereas in 2009, it referred to platelet activation through toll-like receptors.[4] Additionally, thromboinflammation has been used to describe the underlying causes of stroke, which is considered a thromboinflammatory disease.[5]

Today, the relationship between immunothrombosis and thromboinflammation is commonly understood as a cause and effect relationship. Immunothrombosis refers to the influence of the immune system on the formation of a thrombus, whereas thromboinflammation refers to the impact of the thrombus on the immune system.

Immunothrombosis, however, is no prerequisite for thromboinflammation, as nonimmunologic stimuli can also trigger thrombi and subsequently lead to thromboinflammation. While sepsis and ischemia–reperfusion are well known to cause microvascular thrombi that fuel inflammatory processes, other diseases are also associated with thromboinflammatory events including preeclampsia, viral and bacterial infections, as well as hemolytic diseases like sickle cell disease.[6,7] Importantly, certain cancers bear a high risk for immunothrombotic events as well, and the hemostatic system has been demonstrated to regulate several aspects of tumor-associated pathologies.[8,9] Autoimmune diseases, such as rheumatoid arthritis[10] and antiphospholipid syndrome,[11] also trigger immunothrombosis, with thrombi and activated platelets, in turn, exacerbating inflammation in autoimmune conditions.

Notably, thromboinflammation is also critically involved in acute ischemic stroke and coronavirus disease 2019

(COVID-19). Infections triggered by pathogen-associated molecular patterns (PAMPs) and sterile inflammatory diseases caused by damage-associated molecular patterns (DAMPs) can lead to thromboinflammatory states. DAMPs are also released upon tissue injury in situations of ischemia and reperfusion, contributing to thrombus formation. PAMPs and DAMPs interfere with the antithrombotic and anti-inflammatory mechanisms of the vasculature and trigger the dysregulated activation of coagulation factors, platelets, endothelial cells, and complement system. These processes collaborate and intertwine, resulting in surface expression of P-selectin on platelets and endothelium, which facilitates recruitment of leukocytes and prompts a thromboinflammatory state. Furthermore, enhanced release of leukocyte extracellular vesicles (EVs) containing tissue factor (TF) and formation of neutrophil extracellular traps (NETs) leading to procoagulant extracellular DNA can trigger a thrombotic response.[12] These processes not only determine the fate of the local microenvironment, where they lead leads to microthrombi and vessel occlusion, but also have systemic long-term effects beyond ischemic events and can even cause a state of immunosuppression. Widespread tissue damage resulting from stroke, trauma, or burns often leads to a phase of weakened immunity, making the individual more prone to systemic infection, which represents a significant contributor to mortality following sterile tissue injuries.

Cell-free double-stranded DNA released during tissue injury was recently shown to promote this immunosuppressive state, as it causes upregulation of interleukin (IL) 1β, which drives differentiation of CD95L+ myeloid cells and thereby causes postinjury T cell apoptosis and diminished immune responses.[13]

While the causes and consequences of immunothrombosis have been extensively reviewed elsewhere,[1,2,14] this review focuses on the effects of the thrombus on immune function and inflammation.

Inflammation-Induced Coagulation and Coagulation-Mediated Inflammation

Upon vascular injury, the coagulation cascade is initiated by TF. Under quiescent conditions, TF is expressed in an encrypted form on the cell membrane of fibroblasts, pericytes, and vascular smooth muscle cells in subendothelial tissue.[15–17] Upon inflammatory processes, monocytes express TF, thereby inducing coagulation within the circulation and causing immunothrombotic events.[2,18] In addition to being membrane associated, TF is also found on EVs shed from monocytes, which further contributes to thrombus formation.[19] Activated platelets play a pivotal role in the decryption of TF, as they secrete protein disulfide isomerase and foster its activity in response to interaction with monocytes.

TF interacts with and activates FVII to FVIIa, which then forms a complex with phospholipids and Ca^{2+} that activates FX, a central hub of the coagulation cascade where different pathways of induction converge. Coagulation can also be triggered by the so-called contact activation system, which describes the interaction and activation of FXII, FXI, prekallikrein, and their cofactor high molecular weight kininogen by negatively charged surface, e.g., activated platelets expressing phosphatidylserine. FXIIa catalyzes the formation of FXIa from FXI, which then activates FIX to FIXa. FIXa then forms a complex with its cofactor FVIIIa and phospholipids that activates FX.

Of note, not only TF expression by monocytes or endothelial cells carries a procoagulant potential but also extracellular DNA, released as extracellular traps (ETs), can initiate the coagulation cascade via the contact pathway, which represents another central mechanism in immunothrombosis. While neutrophils are the most studied cells to form ETs, other cell types, like eosinophils, macrophages, and mast cells represent a source for extracellular DNA, thereby contributing to immunothrombosis.[20,21]

Together with its cofactor FVa, FXa cleaves prothrombin (FII) to thrombin (FIIa), which catalyzes the cleavage of fibrinogen (FI) to fibrin (FIa). Moreover, thrombin activates FXI, FVIII, and FV, promoting a positive feedback loop. FXIII is also activated by thrombin and crosslinks fibrin to an insoluble fibrin network.

Thrombin and other proteases of the coagulation cascade, including FXa and FVIIa, not only mediate fibrin generation but also regulate cellular functions, which affect hemostatic and inflammatory processes. Proteases can activate G protein-coupled protease-activated receptors (PARs) through an irreversible proteolytic event that results in the generation of a tethered ligand that cannot diffuse away. There are four members of the PAR family. Thrombin activates PAR1, PAR3, and PAR4, whereas multiple trypsin-like serine proteases activate PAR2. Signaling regulation by PAR1 has been extensively studied, but less is known about the other PARs. It has been demonstrated that rapid termination of PAR1 signaling is crucial in determining the cellular protease response's magnitude and kinetics.[22] PAR1 is also the main receptor for thrombin on platelets and represents the most potent pathway of platelet activation. Therefore, PAR1 inhibitors are also clinically used as antiplatelet agents. However, leukocytes also express PARs, inducing proinflammatory and proapoptotic responses, and PARs also modulate stem cell functions via enhanced mobilization of long-term repopulating hematopoietic stem cells.[23]

Endothelial cells express all four PARs and can be activated by FXa, FVIIa, thrombin, activated protein C, and plasmin (reviewed in[24]). PAR signaling leads to endothelial expression of adhesion markers and proinflammatory cytokines and reduces nitric oxide production. PAR signaling has therefore been suggested to contribute to endothelial dysfunction.[25]

Another central mechanism of immunothrombosis is the formation of NETs, which represents a specialized way of controlled cellular death. Of note, platelets are not only activated by immunothrombosis, but can also initiate these processes, e.g., binding of platelets to neutrophils represents a threshold switch for NET formation.[26]

During NET formation, neutrophils not only expel their chromatin, which forms an insoluble net-like DNA structure that is studded with histones and antimicrobial enzymes (e.g., neutrophil elastase) but also retains procoagulant proteins (e.g., von Willebrand factor [vWF], FXI, FXII). Thus, NET formation during infection serves two purposes: on one side, NETs capture and kill bacteria and provide a platform where leukocytes can act more efficiently, which may help to lower bacterial burden.[27–29] On the other side, NETs also serve as a strong prothrombotic and procoagulant stimulus by providing a negatively charged surface that captures coagulation factors and platelets.[29] Histones and immobilized vWF on NETs activate platelets, while negatively charged DNA can trigger the activation of the coagulation cascade via FXIIa-induced thrombin generation (see details above).[30] Additionally, neutrophil elastase on NETs inactivates anticoagulant mechanisms via cleavage of thrombomodulin and tissue factor pathway inhibitor.[1] NET release is a tightly regulated process involving NADPH oxidases and protein arginine deiminase type 4 (PAD4) converting arginine residues to citrulline.[31] Inhibition of PAD4 was demonstrated to be sufficient to prevent NETosis of human and mouse neutrophils.[32] However, PAD4 seems to be only one of several pathways able to induce NET formation as also PAD4-independent mechanisms are described.[31,33] Interestingly, the propensity to form NETs might be an intrinsic property of neutrophil subpopulations, as aging neutrophils form NETs more frequently than young neutrophils and possess higher phagocytic function.[34–36] The central physiological role of NETs in bacterial defense is most evident as various pathogenic bacteria have developed mechanism to circumvent NET formation or reduce NET function.[37]

FXII also exerts proinflammatory responses in leukocytes and endothelial cells and contributes to vascular permeability by increasing endothelial dysfunction, immune cell trafficking,

and mitogenic activity.[38] FXIIa can bind to urokinase-type plasminogen activator receptor on endothelial cells in a platelet-dependent manner and thereby promote activation of the kallikrein–kinin system, which influences inflammation and blood pressure.[38] NETs-driven FXIIa has been suggested to contribute to COVID-19-associated thromboinflammation, and inhibition of FXIIa has been shown to reduce NETs, IL-6 levels, and complement activation in a sepsis model.[39]

The final product of coagulation, fibrin, acts as a scaffold for immune cells and aids in the recruitment and activation of inflammatory cells. Excessive fibrin deposition can lead to fibrosis and chronic inflammation. The impact of coagulation on immune cell functions is summarized in ►Fig. 2.

The Influence of Inflammation on the Fibrinolytic System and How it Feeds Back

The fibrinolytic system, responsible for breaking down blood clots, plays a crucial role in regulating inflammation and maintaining a balance between proinflammatory and anti-inflammatory processes. Its key role in immunomodulation involves clearing of proinflammatory fibrin.

Activated by tissue plasminogen activator (tPA) and urokinase (uPA), the fibrinolytic system ensures effective resolution of the fibrin clot, restoring blood flow after vessel injury. Endothelial cells, urinary epithelial cells, and monocytes/macrophages release tPA and uPA, converting plasminogen into plasmin, the major fibrinolytic enzyme. Fibrin serves as the primary substrate for plasmin, facilitating the interaction of tPA with plasminogen on its surface and thus promoting its own degradation. Plasmin binding to fibrin also protects plasmin from rapid inactivation by α2-antiplasmin.

Carboxypeptidase thrombin activatable fibrinolysis inhibitor (TAFI) can counteract fibrinolysis by removing plasmin binding sites, slowing down plasmin generation, and linking coagulation to fibrinolysis. During fibrinolysis, multiple fibrin degradation products, such as D-dimer and fibrinopeptide B, are released, which possess immunomodulatory and chemotactic functions.

Plasminogen activator inhibitor-1 (PAI-1) and PAI-2, serpins present in plasma, immediately inhibit tPA and uPA, giving them a short half-life. Endothelial cells synthesize PAI-1 and its release is increased in response to inflammatory cytokines. However, PAI-1 is mostly stored in platelets, which also derive PAI-1 from megakaryocytes.

Another serpin that inhibits both tPA and thrombin is protease nexin-1 (PN-1). While it is barely detectable in plasma, it is stored within the α-granules of platelets and released during activation.[40] Platelet PN-1 has the capacity to inhibit both exogenous and endogenous tPA-mediated fibrinolysis as well as platelet activation via inhibition of thrombin.[41]

Various cell types, including endothelial cells, monocytes, macrophages, and neutrophils, participate in fibrinolysis. They express cell surface receptors with fibrinolytic activity, acting as cofactors for plasmin generation and protecting against circulating fibrinolysis inhibitors.

Fibrinolytic proteins also play a crucial role in regulating the immune response. PAI-1 is also an acute phase protein, which is upregulated to protect against bacterial pathogens by promoting bacterial clearance and thereby limiting inflammation.[42] PAI-1 also facilitates neutrophil migration and regulates interferon γ (IFN-γ) responses,[43] whereas PAI-2 dampens proteolytic activity of neutrophils and macrophages.[44]
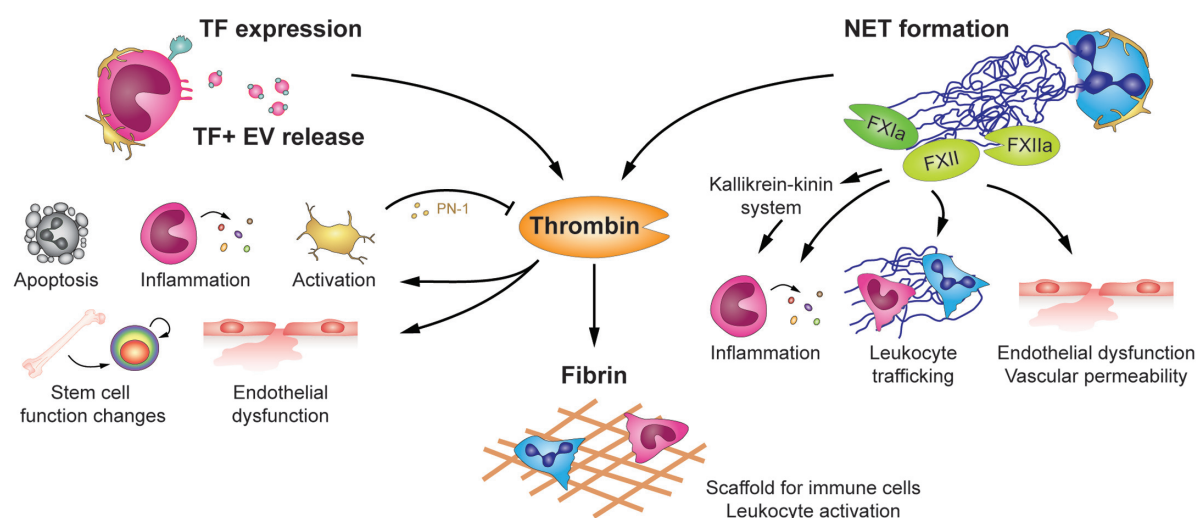

Fig. 2 Impact of coagulation on immune cell functions. Immune-mediated coagulation can be triggered by TF expression or release of TF-positive EVs from monocytes, leading to the formation of thrombin, which subsequently catalyzed the cleavage of fibrinogen to fibrin. By engaging PARs, thrombin also regulates activity and inflammatory responses of platelets, leukocytes, and endothelial cells, but thrombin also exerts proapoptotic effects and enhances hematopoiesis. Thrombin generation can also be induced by NETs, which provide a negatively charged surface that is studded with procoagulant proteins, e.g., FXII. Thereby, NET-bound coagulation factors elicit proinflammatory responses, which may involve activation of the kallikrein–kinin system. In addition, FXII(a) augments leukocyte mobility and contributes to endothelial dysfunction, leading to enhanced vascular permeability. Finally, the fibrin mesh generated by coagulation acts as scaffold for immune cells and aids in their activation and recruitment. EV, extracellular vesicles, FXII(a), (activated) coagulation factor XII; NET, neutrophil extracellular trap; PAR, protease-activated receptor; PN-1: protease nexin-1; TF, tissue factor.
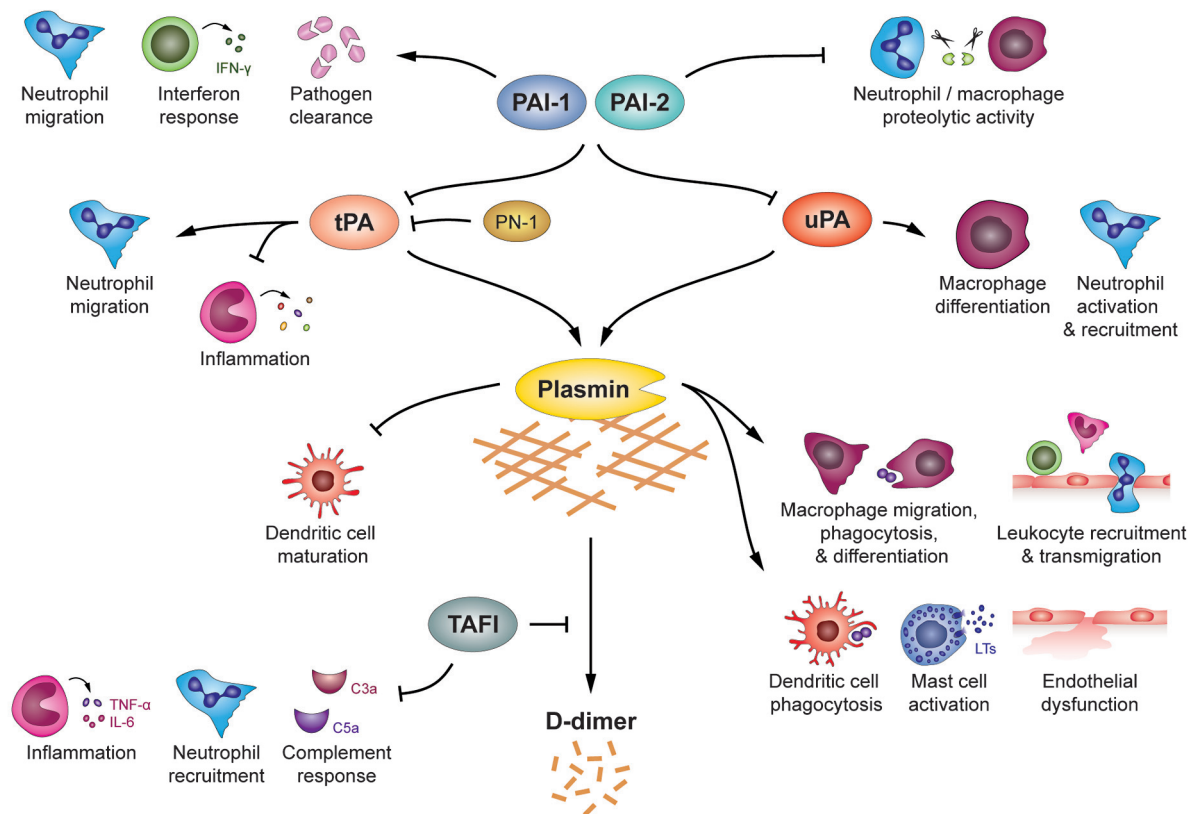
Fig. 3 Immunomodulatory roles of the fibrinolytic system. Fibrinolysis is a tightly controlled system subject to multiple layers of regulation, but the involved proteins also have immunomodulatory effects that may be independent of their (anti-)fibrinolytic function. Plasmin, the central enzyme of fibrinolysis, is activated by tPA and uPA and degrades the fibrin mesh into soluble fibrin degradation products (e.g., D-dimer). Exaggerated fibrinolysis is prevented by PAI-1 and PAI-2, which inhibit both tPA and uPA and thus determine initial plasmin activation, or by TAFI, which impairs subsequent plasmin-mediated fibrin degradation. While TAFI has anti-inflammatory effects on leukocytes and downregulates complement responses, plasmin, uPA, and, in part, also tPA foster proinflammatory responses, e.g., by enhancing activation, differentiation, and recruitment of various leukocyte subsets. Plasmin and PAI-1 also facilitate pathogen clearance by promoting the phagocytic capacity of macrophages and dendritic cells or by augmenting interferon response of T-cells, respectively, which, in turn, may limit pathogen-induced inflammation. C3a, complement component 3a; IFN-γ, interferon γ; IL-6, interleukin 6; LT, leukotriene; PAI, plasminogen activator inhibitor; PN-1: protease nexin-1; TAFI, thrombin-activatable fibrinolysis inhibitor; TNF-α, tumor necrosis factor α; tPA, tissue plasminogen activator; uPA, urokinase.

Plasminogen activators, including tPA and urokinase, modulate the innate immune response, with actions both dependent and independent of their fibrinolytic activity. Urokinase enhances monocyte differentiation into macrophages and promotes neutrophil activation and migration,[44] whereas tPA was found to downregulate inflammation but foster neutrophil adhesion in ischemia/reperfusion and stroke models.[45]

Plasmin(ogen) has diverse roles in regulating proinflammatory processes. It is essential for efficient recruitment of monocytes and lymphocytes during inflammation and promotes macrophage phagocytosis, migration, and differentiation (reviewed in[46]). In ischemia/reperfusion and stroke models, tPA-mediated plasmin activity was also critical for neutrophil transmigration and disruption of endothelial junctions,[45] while mast cell activation and leukotriene generation were required for neutrophil recruitment.[45] In addition, plasmin enhances dendritic cell phagocytosis, keeping them in an immature phenotype and reducing migration to lymph nodes.[47]

TAFI modulates inflammation by removing specific residues from various inflammatory mediators, including C3a and C5a,[48] dampening neutrophil recruitment and reducing

TNF-α and IL-6 levels independently of its antifibrinolytic function.[49]

The fibrinolytic system's varying roles in immunomodulation, summarized in ►Fig. 3, highlight complex interactions that must be carefully balanced to avoid exacerbating inflammatory responses and promoting a prothrombotic environment.

Platelets Modulate Leukocyte Functions and Leukocytes Modulate Platelet Fate and Production

Upon activation, platelets quickly adhere to leukocytes through interactions facilitated by platelet CD62P and leukocyte P-selectin glycoprotein ligand-1. Monocytes exhibit the highest affinity for CD62P, followed by granulocytes and lymphocytes. This initial binding is stabilized by a variety of other receptors (reviewed in[50]) and leads to the targeted release of soluble mediators, mutual activation, and fine-tuning of immune and inflammatory responses (►Fig. 4).

Beyond direct interaction, platelets can also release EVs to communicate with leukocytes. This communication involves
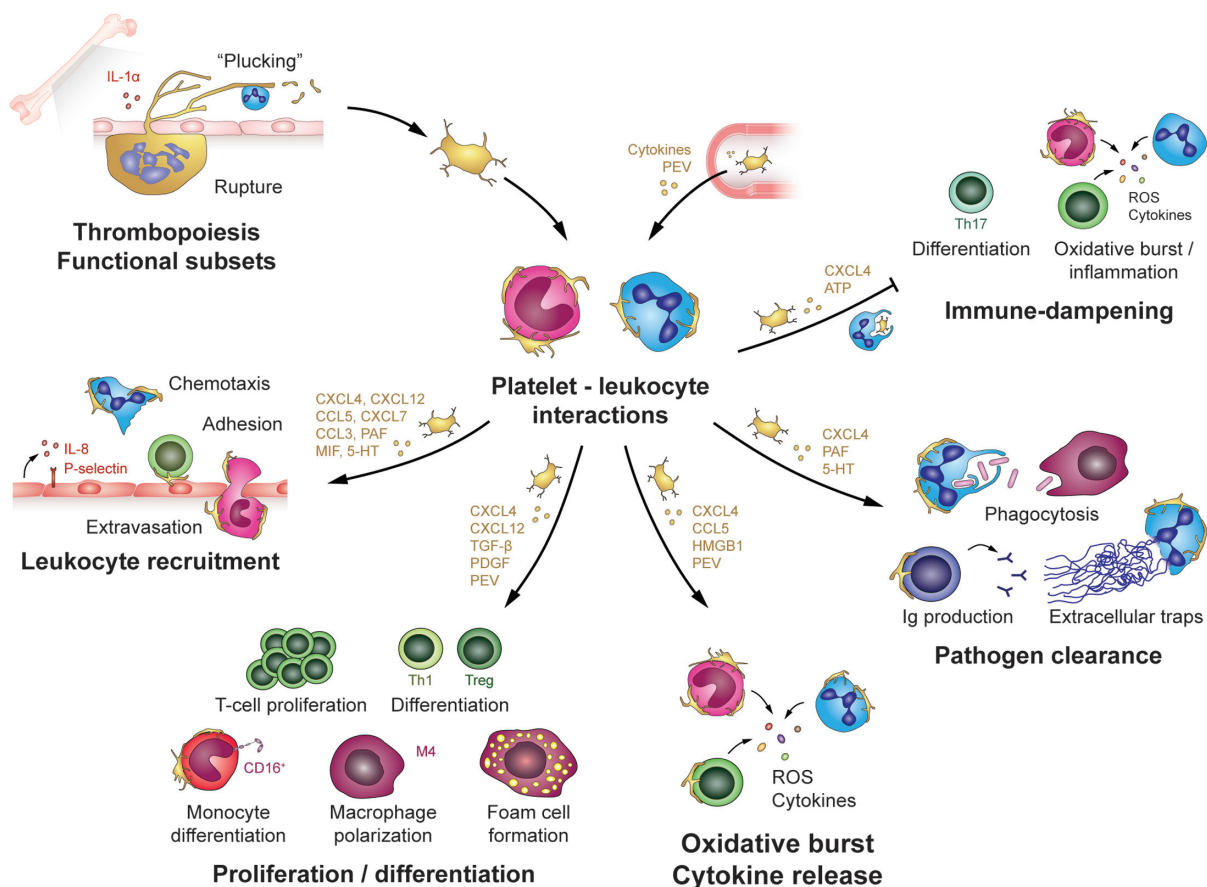
Fig. 4 The role of platelets in thromboinflammation. The immune system plays an important role in thrombopoiesis as neutrophils pluck on proplatelets to assist in their release from megakaryocytes, thereby contributing to platelet production under physiological conditions. Under inflammatory conditions, mediators such as IL-1α can further stimulate thrombopoiesis, which may also convey distinct characteristics to newly produced platelets. Upon their release into the circulation, platelets can interact with leukocytes either directly via cell–cell adhesion or indirectly via release of cyto-/chemokines or PEVs, which allow platelets to modulate immune responses even at distant sites. Thereby, platelets fine-tune various proinflammatory immune responses, including leukocyte migration and recruitment, which is also indirectly facilitated by platelet-stimulated endothelial activation. In addition, platelets contribute to proliferation and differentiation of T-cells and are important determinants for monocytes/macrophages polarization/differentiation into proinflammatory and proatherogenic subtypes. Platelet–leukocyte interplay can further trigger an oxidative burst as well as modulate quality and quantity of released cytokines. In infectious setting, platelet-mediated immunomodulation also supports pathogen clearance, by augmenting pathogen phagocytosis and antibody production as well as inducing NETs. However, under certain conditions, platelet–leukocyte interactions may also dampen immune responses, e.g., by preventing lymphocyte differentiation, interfering with neutrophil ROS production, or altering monocyte-derived cyto-/chemokines toward a more anti-inflammatory profile. 5-HT, serotonin; ATP, adenosine triphosphate; CCL5, chemokine (C–C motif) ligand 5; CXCL4, chemokine (C–X–C motif) ligand 4; HMGB1, high mobility group box 1; Ig, immunoglobulin; IL-1α, interleukin 1α; MIF, macrophage migration inhibitory factor; NET, neutrophil extracellular trap; PAF, platelet activating factor; PDGF, platelet-derived growth factor; PEV, platelet-derived extracellular vesicles; ROS, reactive oxygen species; TGF-β, transforming growth factor β; Th1, T helper cell type 1; Treg, regulatory T cell.

receptor-mediated signaling and transfer of proteins or nucleic acids (as reviewed in[51]). Through the release of soluble mediators, platelets can further modulate leukocyte functions at distant sites.

Platelets play a significant role in enhancing immune responses and leukocyte functions. Platelet-derived chemokines, such as platelet factor 4 (PF4/CXCL4), increase endothelial adhesion of neutrophils and monocytes. They indirectly promote leukocyte migration through endothelial cell activation, induced by platelet-derived serotonin (5-HT), which leads to endothelial CD62P expression and IL-8 release, triggering leukocyte rolling, adhesion, and extravasation.

Moreover, platelets enhance leukocyte activation, resulting in increased cytokine release and oxidative burst in neutrophils. They also promote pathogen clearance by neutrophils and contribute to NET formation. Platelets influence monocyte differentiation, favoring the switch to CD16-positive monocytes. Furthermore, platelet activation and CXCL4 release are associated with macrophage phenotype switches. Thereby, platelets also facilitate foam cell formation and contribute to atherogenesis.[52]

Although platelet–leukocyte interplay generally promotes inflammation, there are also some reports that suggest an anti-inflammatory role of platelet–leukocyte interplay. For example, annexin A1 selectively modifies platelet surface determinants, including phosphatidylserine, to promote platelet phagocytosis by neutrophils, thereby leading to anti-inflammatory effects and actively driving thrombus resolution.[53]

Platelets also play a crucial role in lymphocyte trafficking to secondary lymphoid organs and have a significant impact on the function and differentiation of T-lymphocytes and B-lymphocytes. This highlights their essential role in regulating and modulating adaptive immune responses.[20]

Increasing evidence suggests an intricate interaction between inflammation and platelet production. In inflammatory conditions, IL-1α triggers rapid megakaryocyte rupture-dependent thrombopoiesis, leading to elevated platelet counts.[54] Additionally, neutrophils play a direct role in accelerating proplatelet growth through plucking, thereby facilitating continuous platelet production.[55] These processes might endow platelets with functional attributes right from their inception. However, further research is needed to precisely understand the role of inflammatory drivers in platelet production and their contributions to distinct platelet subsets.

Complement

The complement system, which is a crucial part of the immune system in defense against pathogens and for removal of damaged cells, consists of a complex network of plasma proteins that interact in a cascade-like manner. The complement system can be activated through three main pathways: the classical pathway, the alternative pathway, and the lectin pathway. Once activated, the complement system generates a series of reactions, leading to the formation of membrane attack complexes (MACs) on the surface of pathogens or infected cells that can destroy invading pathogens, enhance phagocytosis by immune cells, and trigger inflammation.

The complement system and platelets have intricate interactions with each other, influencing immune responses and coagulation. Complement proteins can initiate a cascade of reactions upon binding to platelet receptors, resulting in the recruitment and activation of immune cells at the site of injury or infection. Platelets express various complement receptors (cC1qR, gC1qR, C3aR, and C5aR), whereas complement components (C1q, C3, C4, and C9) can bind to activated platelet surfaces.[56] This binding activates platelets and leads to the surface expression of P-selectin, which facilitates neutrophil adhesion to the endothelium.[57] Megakaryocytes and platelets store C3 in their granules, releasing it upon activation.[58]

Platelet-associated factors like chondroitin sulfate A or phosphatidylserine exposure can initiate the complement cascade via C1q/r/s,[56] whereas P-selectin and properdin stabilize C3 and C5 convertases.[59] The subsequent formation of MACs leads to the release of procoagulant EVs from platelets and endothelial cells.[60]

Platelet-derived ATP and Ca^{2+} also contribute to the extracellular phosphorylation of C3 and its fragments, prolonging C3b activity and amplifying complement activation,[61] whereas platelets also express complement control proteins like CD55, CD59, and factor H to regulate complement activation on their surface to prevent overshooting responses.[62]

The complement system not only interacts with primary hemostasis but also with secondary hemostasis, as both cascades share activators and inhibitors. FXIIa can activate C1q, thereby initiating the classical pathway.[63] C1q esterase inhibitor interferes with all three complement pathways as well as FXIIa-mediated coagulation.[59] Thrombin can cleave C3 and C5, further amplifying complement activation.[60,64]

In turn, complement anaphylatoxins (C3a, C4a, C5a) can directly or indirectly activate innate immune cells and stimulate endothelial release of proinflammatory mediators like IL-6, IL-8, and CCL2.[65] In particular, C5a upregulates TF and PAI-1 expression on neutrophils and endothelial cells[59,66] and also mediates vWF secretion from endothelial cells, while C5b induces TF expression on monocytes.[65,67] These interactions illustrate the complex crosstalk between the complement system, platelets, coagulation, and immune responses (▶Fig. 5).

Conclusion

Despite the growing body of evidence that sheds light on how the immune system triggers thrombotic events, our understanding of how the hemostatic system reciprocally influences inflammatory and immune responses remains limited. The complexity of these interactions is amplified by the profound sensitivity of the hemostatic system to factors such as the underlying disease context, the specific anatomical localization, and the current state of the disease process.

These dynamic interactions between thrombosis and inflammation can therefore take on a dual nature, acting either as allies or adversaries depending on the disease context. In some cases, the involvement of hemostatic elements in immune responses can be advantageous, aiding in the containment and resolution of infections or injuries. Conversely, under different circumstances, these same mechanisms can exacerbate inflammatory processes and foster pathological conditions.

The significant gap in our understanding of thromboinflammation highlights a critical deficiency in therapeutic approaches, leaving us without precise targets necessary to effectively address the inflammatory dimension of thrombosis. Extensive research over the last decade has led to a myriad of innovative approaches directed against immunothrombosis. These strategies aim to avert potential thrombotic events in high-risk patients without increasing their risk of bleeding.

Targeting the formation of NETs stands as a promising therapeutic approach. Degradation of NETs provides a safe treatment option, as seen with drugs like DNase I, which is already employed in diseases like cystic fibrosis and off-label for COVID-19.[68,69] Additionally, the administration of heparin or colchicine offers possibilities to disrupt NET formation by inhibiting histone-induced coagulation or actin cytoskeleton rearrangement in NET-forming neutrophils, respectively.[70,71]

Finally, novel inhibitors that interfere with myeloperoxidase or PAD4 represent potential therapeutic drugs to limit NET formation, validated in various inflammatory disease models such as hepatic ischemia/reperfusion injury, vasculitis, and systemic lupus erythematosus.[72–74]

Various new antiplatelet agents are currently under examination to prevent inflammation-induced thrombus formation. Alternative strategies involve impeding platelet–leukocyte interactions, blocking alternative surface receptors,[75] or
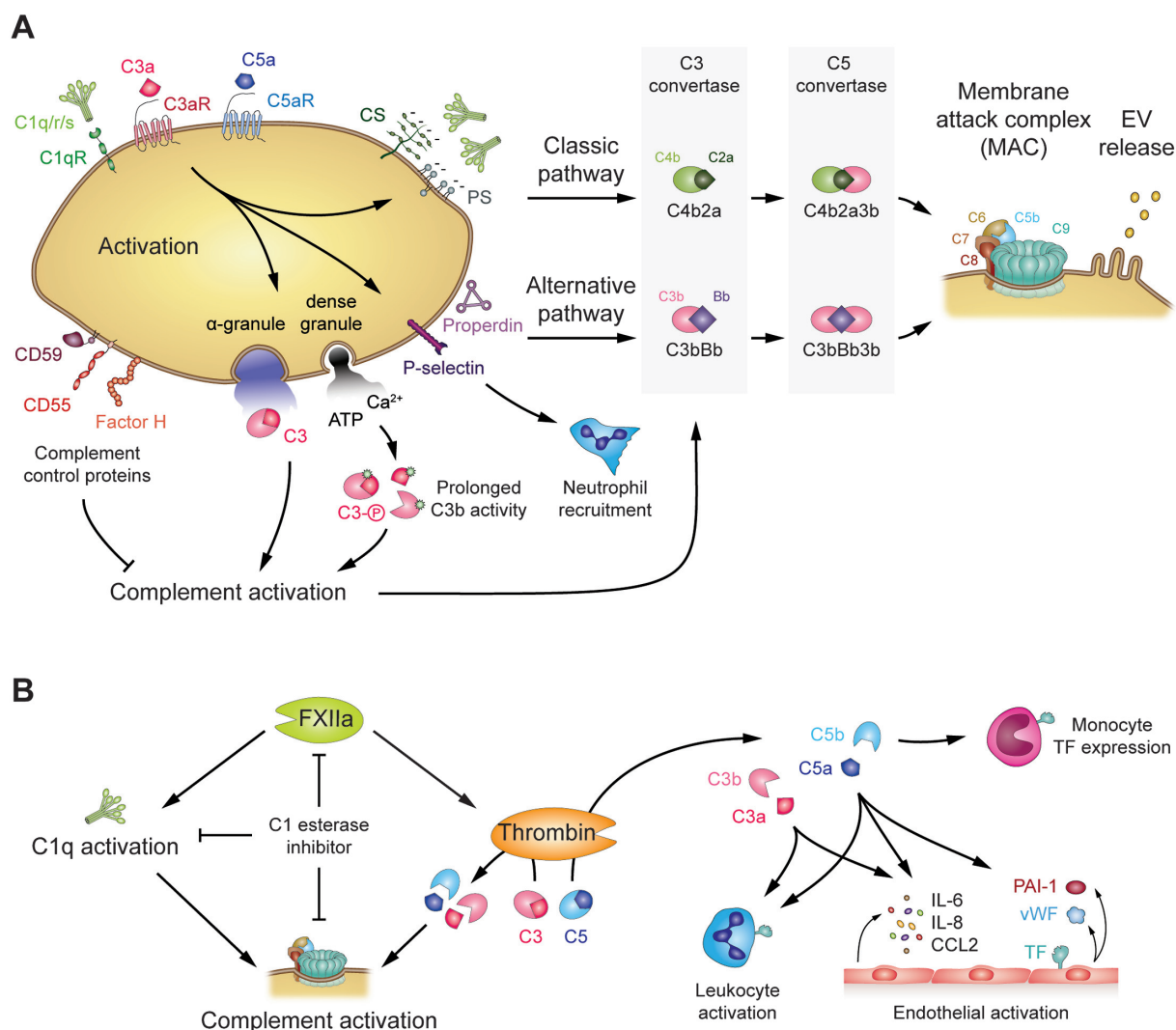
Fig. 5 Complement interplay with the hemostatic system. (**A**) Binding of complement factors to respective receptors on platelets leads to their activation, inducing surface expression of PS and P-selectin, which is essential for platelet–leukocyte binding and subsequent immunomodulation. Negatively charged CS and PS can initiate the classic pathway, whereas P-selectin and properdin mediate the alternative pathway, both of which result in MAC formation on and EV release from target cells, such as platelets or endothelial cells. Activated platelets also release C3 from their α-granules, whereas dense granule-derived Ca^{2+} and ATP mediate phosphorylation of C3 and its fragments, which further amplifies complement activation. At the same time, platelets express complement control proteins that prevent overshooting complement responses. (**B**) Activated coagulation factors FXIIa and thrombin can trigger the complement system by activating C1q or by cleaving C3 and C5, promoting all three complement pathways. Notably, the serpin C1 esterase inhibitor targets components of all three complement pathways as well as FXIIa to regulate both complement and coagulation responses. C3a and C5a also convey proinflammatory and procoagulant effects by activating leukocyte and endothelial cells and inducing TF expression and release of procoagulant vWF or antifibrinolytic PAI-1. ATP, adenosine triphosphate, C1q, complement component 1q; C1qR, C1q receptor; CCL2, chemokine (C–C motif) ligand 2; CS, chondroitin sulfate A; EV, extracellular vesicle; FXIIa, activated coagulation factor XII; IL-6, interleukin 6; MAC, membrane attack complex; PAI-1, plasmin activator inhibitor 1; PS, phosphatidylserine; TF, tissue factor; vWF, von Willebrand factor.

inhibition of intracellular signaling pathways such as immunoreceptor tyrosine-based activation motif signaling or phosphoinositide 3 kinase signaling.[76–78] Likewise, novel methods to inhibit the coagulation cascade are in development. FXI inhibitors show promising results in clinical trials[79] and also strategies for FXII inhibition are currently under investigation[80].

Current studies are exploring and expanding the utilization of intra-arterial thrombolysis subsequent to mechanical thrombectomy to initiate tissue reperfusion in acute ischemic stroke. Tenecteplase, a genetically modified recombi-

nant tPA with increased fibrin specificity, has been proven to be quicker and more efficient compared with commonly used Alteplase (recombinant tPA).[81] Further, a recent trial revealed promising results in shielding the brain from tissue damage, thereby preventing death and disability by the TLR4 antagonist ApTOLL.[82]

Closing this knowledge gap in the near future is of crucial importance, as it holds the potential to unveil novel strategies for therapeutic intervention, ultimately leading to more precise and effective treatments for thrombotic disorders with inflammatory components.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1 Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol 2013;13(01):34–45

2 Gaertner F, Massberg S. Blood coagulation in immunothrombosis-at the frontline of intravascular immunity. Semin Immunol 2016; 28(06):561–569

3 Tanguay JF, Geoffroy P, Sirois MG, et al. Prevention of in-stent restenosis via reduction of thrombo-inflammatory reactions with recombinant P-selectin glycoprotein ligand-1. Thromb Haemost 2004;91(06):1186–1193

4 Blair P, Rex S, Vitseva O, et al. Stimulation of Toll-like receptor 2 in human platelets induces a thromboinflammatory response through activation of phosphoinositide 3-kinase. Circ Res 2009; 104(03):346–354

5 Nieswandt B, Kleinschnitz C, Stoll G. Ischaemic stroke: a thrombo-inflammatory disease? J Physiol 2011;589(17):4115–4123

6 Dimitrov JD, Roumenina LT, Perrella G, Rayes J. Basic mechanisms of hemolysis-associated thrombo-inflammation and immune dysregulation. Arterioscler Thromb Vasc Biol 2023;43(08): 1349–1361

7 Conran N, De Paula EV. Thromboinflammatory mechanisms in sickle cell disease - challenging the hemostatic balance. Haematologica 2020;105(10):2380–2390

8 Palacios-Acedo AL, Mège D, Crescence L, Dignat-George F, Dubois C, Panicot-Dubois L. Platelets, thrombo-inflammation, and cancer: collaborating with the enemy. Front Immunol 2019;10:1805

9 Stoiber D, Assinger A. Platelet-leukocyte interplay in cancer development and progression. Cells 2020;9(04):855

10 Olumuyiwa-Akeredolu OO, Page MJ, Soma P, Pretorius E. Platelets: emerging facilitators of cellular crosstalk in rheumatoid arthritis. Nat Rev Rheumatol 2019;15(04):237–248

11 Müller-Calleja N, Hollerbach A, Royce J, et al. Lipid presentation by the protein C receptor links coagulation with autoimmunity. Science 2021;371(6534):eabc0956

12 Swystun LL, Liaw PC. The role of leukocytes in thrombosis. Blood 2016;128(06):753–762

13 Roth S, Cao J, Singh V, et al. Post-injury immunosuppression and secondary infections are caused by an AIM2 inflammasome-driven signaling cascade. Immunity 2021;54(04):648–659.e8

14 Stark K, Massberg S. Interplay between inflammation and thrombosis in cardiovascular pathology. Nat Rev Cardiol 2021;18(09): 666–682

15 Fleck RA, Rao LV, Rapaport SI, Varki N. Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. Thromb Res 1990;59(02):421–437

16 Mackman N, Taubman MB. Does tissue factor expression by vascular smooth muscle cells provide a link between C-reactive protein and cardiovascular disease? Arterioscler Thromb Vasc Biol 2008;28(04):601–603

17 McDonald AG, Yang K, Roberts HR, Monroe DM, Hoffman M. Perivascular tissue factor is down-regulated following cutaneous wounding: implications for bleeding in hemophilia. Blood 2008; 111(04):2046–2048

18 Cirillo P, Golino P, Calabrò P, et al. C-reactive protein induces tissue factor expression and promotes smooth muscle and endothelial cell proliferation. Cardiovasc Res 2005;68(01):47–55

19 Massberg S, Grahl L, von Bruehl ML, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat Med 2010;16(08):887–896

20 Schrottmaier WC, Mussbacher M, Salzmann M, Assinger A. Platelet-leukocyte interplay during vascular disease. Atherosclerosis 2020;307:109–120

21 Shi C, Yang L, Braun A, Anders HJ. Extracellular DNA-a Danger signal triggering immunothrombosis. Front Immunol 2020;11:568513

22 Trejo J. Protease-activated receptors: new concepts in regulation of G protein-coupled receptor signaling and trafficking. J Pharmacol Exp Ther 2003;307(02):437–442

23 Gur-Cohen S, Itkin T, Chakrabarty S, et al. PAR1 signaling regulates the retention and recruitment of EPCR-expressing bone marrow hematopoietic stem cells. Nat Med 2015;21(11):1307–1317

24 Chandrabalan A, Ramachandran R. Molecular mechanisms regulating proteinase-activated receptors (PARs). FEBS J 2021;288 (08):2697–2726

25 Minami T, Sugiyama A, Wu SQ, Abid R, Kodama T, Aird WC. Thrombin and phenotypic modulation of the endothelium. Arterioscler Thromb Vasc Biol 2004;24(01):41–53

26 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

27 Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303(5663):1532–1535

28 McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. Cell Host Microbe 2012;12(03):324–333

29 von Brühl ML, Stark K, Steinhart A, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J Exp Med 2012;209(04):819–835

30 Rangaswamy C, Englert H, Deppermann C, Renné T Polyanions in coagulation and thrombosis: focus on polyphosphate and neutrophils extracellular traps. Thromb Haemost 2021;121(08):1021–1030

31 Kenny EF, Herzig A, Krüger R, et al. Diverse stimuli engage different neutrophil extracellular trap pathways. eLife 2017;6:x

32 Lewis HD, Liddle J, Coote JE, et al. Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. Nat Chem Biol 2015;11(03):189–191

33 Sollberger G, Tilley DO, Zychlinsky A. Neutrophil extracellular traps: the biology of chromatin externalization. Dev Cell 2018;44 (05):542–553

34 Silvestre-Roig C, Hidalgo A, Soehnlein O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. Blood 2016; 127(18):2173–2181

35 Uhl B, Vadlau Y, Zuchtriegel G, et al. Aged neutrophils contribute to the first line of defense in the acute inflammatory response. Blood 2016;128(19):2327–2337

36 Zhang D, Chen G, Manwani D, et al. Neutrophil ageing is regulated by the microbiome. Nature 2015;525(7570):528–532

37 Meyers S, Crescente M, Verhamme P, Martinod K. *Staphylococcus aureus* and neutrophil extracellular traps: the master manipulator meets its match in immunothrombosis. Arterioscler Thromb Vasc Biol 2022;42(03):261–276

38 Mailer RK, Rangaswamy C, Konrath S, Emsley J, Renné T An update on factor XII-driven vascular inflammation. Biochim Biophys Acta Mol Cell Res 2022;1869(01):119166

39 Jansen PM, Pixley RA, Brouwer M, et al. Inhibition of factor XII in septic baboons attenuates the activation of complement and fibrinolytic systems and reduces the release of interleukin-6 and neutrophil elastase. Blood 1996;87(06):2337–2344

40 Madjene C, Boutigny A, Bouton MC, Arocas V, Richard B. Protease Nexin-1 in the cardiovascular system: wherefore art thou? Front Cardiovasc Med 2021;8:652852

41 Boulaftali Y, Adam F, Venisse L, et al. Anticoagulant and antithrombotic properties of platelet protease nexin-1. Blood 2010;115 (01):97–106

42 Lim JH, Woo CH, Li JD. Critical role of type 1 plasminogen activator inhibitor (PAI-1) in early host defense against nontypeable *Haemophilus influenzae* (NTHi) infection. Biochem Biophys Res Commun 2011;414(01):67–72

43 Renckens R, Pater JM, van der Poll T. Plasminogen activator inhibitor type-1-deficient mice have an enhanced IFN-gamma response to lipopolysaccharide and staphylococcal enterotoxin B. J Immunol 2006;177(11):8171–8176

44 Whyte CS. All tangled up: interactions of the fibrinolytic and innate immune systems. Front Med (Lausanne) 2023;10:1212201

45 Uhl B, Zuchtriegel G, Puhr-Westerheide D, et al. Tissue plasminogen activator promotes postischemic neutrophil recruitment via its proteolytic and nonproteolytic properties. Arterioscler Thromb Vasc Biol 2014;34(07):1495–1504

46 Medcalf RL, Keragala CB. Fibrinolysis: a primordial system linked to the immune response. Int J Mol Sci 2021;22(07):3406

47 Borg RJ, Samson AL, Au AE, et al. Dendritic cell-mediated phagocytosis but not immune activation is enhanced by plasmin. PLoS One 2015;10(07):e0131216

48 Plug T, Meijers JC. Structure-function relationships in thrombin-activatable fibrinolysis inhibitor. J Thromb Haemost 2016;14(04):633–644

49 Renckens R, Roelofs JJ, ter Horst SA, et al. Absence of thrombin-activatable fibrinolysis inhibitor protects against sepsis-induced liver injury in mice. J Immunol 2005;175(10):6764–6771

50 Schrottmaier WC, Kral JB, Badrnya S, Assinger A. Aspirin and P2Y12 Inhibitors in platelet-mediated activation of neutrophils and monocytes. Thromb Haemost 2015;114(03):478–489

51 Mussbacher M, Pirabe A, Brunnthaler L, Schrottmaier WC, Assinger A. Horizontal microRNA transfer by platelets - evidence and implications. Front Physiol 2021;12:678362

52 Kral JB, Schrottmaier WC, Salzmann M, Assinger A. Platelet interaction with innate immune cells. Transfus Med Hemother 2016;43(02):78–88

53 Senchenkova EY, Ansari J, Becker F, et al. Novel role for the AnxA1-Fpr2/ALX signaling axis as a key regulator of platelet function to promote resolution of inflammation. Circulation 2019;140(04):319–335

54 Nishimura S, Nagasaki M, Kunishima S, et al. IL-1α induces thrombopoiesis through megakaryocyte rupture in response to acute platelet needs. J Cell Biol 2015;209(03):453–466

55 Petzold T, Zhang Z, Ballesteros I, et al. Neutrophil "plucking" on megakaryocytes drives platelet production and boosts cardiovascular disease. Immunity 2022;55(12):2285–2299.e7

56 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

57 Peerschke EI, Ghebrehiwet B. Platelet receptors for the complement component C1q: implications for hemostasis and thrombosis. Immunobiology 1998;199(02):239–249

58 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

59 Rawish E, Sauter M, Sauter R, Nording H, Langer HF. Complement, inflammation and thrombosis. Br J Pharmacol 2021;178(14):2892–2904

60 Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD. Interactions between coagulation and complement–their role in inflammation. Semin Immunopathol 2012;34(01):151–165

61 Ekdahl KN, Rönnblom L, Sturfelt G, Nilsson B. Increased phosphate content in complement component C3, fibrinogen, vitronectin, and other plasma proteins in systemic lupus erythematosus: covariation with platelet activation and possible association with thrombosis. Arthritis Rheum 1997;40(12):2178–2186

62 Verschoor A, Langer HF. Crosstalk between platelets and the complement system in immune protection and disease. Thromb Haemost 2013;110(05):910–919

63 Amara U, Rittirsch D, Flierl M, et al. Interaction between the coagulation and complement system. Adv Exp Med Biol 2008;632:71–79

64 Wojta J, Huber K, Valent P. New aspects in thrombotic research: complement induced switch in mast cells from a profibrinolytic to a prothrombotic phenotype. Pathophysiol Haemost Thromb 2003;33(5-6):438–441

65 Foley JH, Conway EM. Cross talk pathways between coagulation and inflammation. Circ Res 2016;118(09):1392–1408

66 Wojta J, Kaun C, Zorn G, et al. C5a stimulates production of plasminogen activator inhibitor-1 in human mast cells and basophils. Blood 2002;100(02):517–523

67 Langer F, Spath B, Fischer C, et al. Rapid activation of monocyte tissue factor by antithymocyte globulin is dependent on complement and protein disulfide isomerase. Blood 2013;121(12):2324–2335

68 Thomson AH. Human recombinant DNase in cystic fibrosis. J R Soc Med 1995;88(Suppl 25, Suppl 25):24–29

69 Holliday ZM, Earhart AP, Alnijoumi MM, Krvavac A, Allen LH, Schrum AG. Non-randomized trial of dornase alfa for acute respiratory distress syndrome secondary to Covid-19. Front Immunol 2021;12:714833

70 Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci U S A 2010;107(36):15880–15885

71 Neeli I, Dwivedi N, Khan S, Radic M. Regulation of extracellular chromatin release from neutrophils. J Innate Immun 2009;1(03):194–201

72 Huang H, Tohme S, Al-Khafaji AB, et al. Damage-associated molecular pattern-activated neutrophil extracellular trap exacerbates sterile inflammatory liver injury. Hepatology 2015;62(02):600–614

73 Zheng W, Warner R, Ruggeri R, et al. PF-1355, a mechanism-based myeloperoxidase inhibitor, prevents immune complex vasculitis and anti-glomerular basement membrane glomerulonephritis. J Pharmacol Exp Ther 2015;353(02):288–298

74 Knight JS, Zhao W, Luo W, et al. Peptidylarginine deiminase inhibition is immunomodulatory and vasculoprotective in murine lupus. J Clin Invest 2013;123(07):2981–2993

75 Prasannan N, Scully M. Novel antiplatelet strategies targeting VWF and GPIb. Platelets 2021;32(01):42–46

76 Vilahur G, Gutiérrez M, Arzanauskaite M, Mendieta G, Ben-Aicha S, Badimon L. Intracellular platelet signalling as a target for drug development. Vascul Pharmacol 2018;111:22–25

77 McFadyen JD, Schaff M, Peter K. Current and future antiplatelet therapies: emphasis on preserving haemostasis. Nat Rev Cardiol 2018;15(03):181–191

78 Schrottmaier WC, Kral-Pointner JB, Salzmann M, et al. Platelet p110β mediates platelet-leukocyte interaction and curtails bacterial dissemination in pneumococcal pneumonia. Cell Rep 2022;41(06):111614

79 Verhamme P, Yi BA, Segers A, et al; ANT-005 TKA Investigators. Abelacimab for prevention of venous thromboembolism. N Engl J Med 2021;385(07):609–617

80 Labberton L, Kenne E, Long AT, et al. Neutralizing blood-borne polyphosphate in vivo provides safe thromboprotection. Nat Commun 2016;7:12616

81 Miller SE, Warach SJ. Evolving thrombolytics: from alteplase to tenecteplase. Neurotherapeutics 2023;20(03):664–678

82 Hernández-Jiménez M, Abad-Santos F, Cotgreave I, et al. Safety and efficacy of ApTOLL in patients with ischemic stroke undergoing endovascular treatment: a phase 1/2 randomized clinical trial. JAMA Neurol 2023;80(08):779–788