Neutrophil-Platelet Interactions as Novel Treatment Targets in Cardiovascular Disease

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Neutrophils and platelets are among the most abundant cell types in peripheral blood and characterized by high plasticity and a readily available reservoir of surface proteins and secretable granule contents. Receptor-mediated activation and granule release predispose both cell types for rapid responses to various stimuli. While neutrophils provide the first line of defense to microbial infections and platelets are known for their aggregatory functions in hemostasis and thrombosis, research of the past decade has highlighted that both cell types jointly shape local and systemic immune responses and clot formation alike. Concomitant activation of neutrophils and platelets has been observed in a variety of cardiovascular diseases, including arterial and venous thrombosis, atherosclerosis as well as myocardial infarction and ischemia-reperfusion injury. In this review, we describe the mechanisms by which neutrophils and platelets interact physically, how release of granule contents and soluble molecules by either cell type affects the other and how this mutual activation supports the efficacy of immune responses. We go on to describe how activated platelets contribute to host defense by triggering neutrophil extracellular trap (NET) formation in a process termed immunothrombosis, which in turn promotes local platelet activation and coagulation. Further, we review current evidence of hazardous overactivation of either cell type and their respective role in cardiovascular disease, with a focus on thrombosis, myocardial infarction and ischemia-reperfusion injury, and describe how neutrophils and platelets shape thromboinflammation in COVID-19. Finally, we provide an overview of therapeutic approaches targeting neutrophil-platelet interactions as novel treatment strategy in cardiovascular disease.

Keywords: neutrophil, platelet, cardiovascular disease, thrombosis, NETosis

INTRODUCTION

Neutrophils and platelets are among the most abundant cells in peripheral blood. Traditionally, a clear division of labor was proposed between these cell types: one providing the most essential of host defenses against invading pathogens (1), the other forming plugs to prevent bleeding (2). Evolutionarily, inflammatory reactions to invading pathogens and hemostatic responses share common features: Both infection and impairment of vascular integrity observed in sterile injury lead to exposure of damage- or pathogen-associated molecular patterns (PAMPs and DAMPs),

respectively, the sensing of which results in immune cell activation, cytokine and granule release and concurrent effects on vascular homeostasis (3, 4). Consequently, the traditional view of platelets and neutrophils acting separately has started to blur, with both clinical and experimental studies pointing toward the involvement of both cell types in coagulation and immunity alike (5, 6). Interestingly, neutrophils and platelets show significant overlap of surface receptors such as Toll-like Receptors (TLR) (1, 7–9) capable of sensing DAMPs and PAMPs, and both possess an intracellular weaponry of granules, ready to be released upon detection of pathogens or loss of endothelial integrity, respectively.

In this review, we describe the armamentarium of both platelets and neutrophils comprising multiple surface receptors and soluble agonists that shape the intricate interplay between both cell types. We go on to describe the effects of reciprocal platelet-neutrophil activation observed in inflammation and its impact on immune responses and clot formation alike. Specifically, we focus on platelet-neutrophil interactions and their hazardous overactivation in cardiovascular disease, with a focus on atherosclerosis, thrombosis, and ischemia-reperfusion injury (IRI). We then provide an overview on how detrimental platelet and neutrophil overactivation and exhaustion drive cardiovascular complications and a prothrombotic phenotype observed in severe Coronavirus disease 2019 (COVID-19). Finally, we summarize currently investigated therapeutic approaches that target platelet-neutrophil interactions.

MECHANISMS OF PLATELET-NEUTROPHIL INTERPLAY

Direct Interactions

P-selectin on platelets and P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils are among the most important molecules mediating platelet-neutrophil interaction. Together with glycoprotein (GP) Ibα and the neutrophil integrin αMβ2, known as Mac-1 (CD11b/CD18), this receptor pair drives platelet-neutrophil interactions under (thrombo)inflammatory conditions (**Figure 1A**) (10–12). Indeed, adherent neutrophils were shown to actively scan flowing blood for activated platelets using PSGL-1-positive uropods to subsequently transmigrate across the inflamed endothelium (11). Mac-1 also mediates neutrophil-platelet interactions through a third receptor expressed on platelets, the junctional adhesion molecule 3 (JAM-3) (13).

Interestingly, P-selectin-induced neutrophil activation supports neutrophilic secretion of cathepsin G (CTSG) and neutrophil elastase (ELANE), both of which cleave the N-terminus of surface PSGL-1, thereby negatively regulating further activation (12). Direct physical interactions between platelets and neutrophils are also mediated by CD40 ligand (CD40L/CD154) and CD40 (14), the adhesion-molecule ICAM-2 and integrin αLβ2, known as Lymphocyte function-associated antigen 1 (LFA-1) (5), and TREM-1 ligand (TREM-1L) and TREM-1 (15) on platelets and neutrophils, respectively (**Figure 1A**). Binding *via* these receptors can induce reciprocal
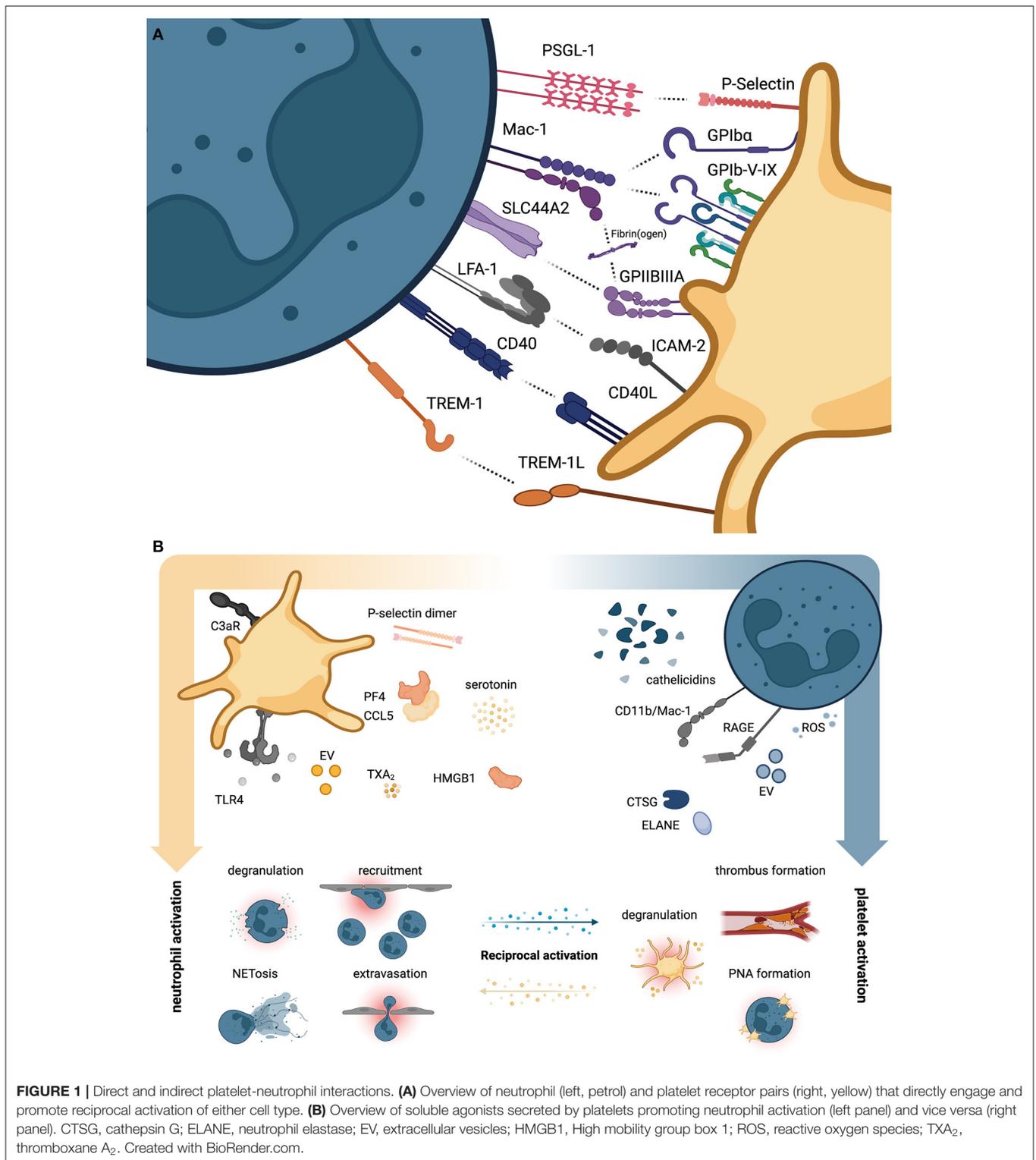
activation of downstream signaling cascades: For e.g., TREM-1L/TREM-1 binding promotes neutrophil reactive oxygen species (ROS) production, degranulation and release of IL-8 (15, 16), without affecting platelet aggregation (15), while CD40-CD40L interaction boosts platelet-neutrophil interaction, platelet activation and enhances neutrophil recruitment through upregulation of Mac-1 (17, 18).

Notably, well-known platelet adhesion receptors GPIb-V-IX complex and integrin αIIbβ3, known as GPIIBIIIA, which mediate binding of extracellular matrix and plasma proteins such as von Willebrand factor (vWF) and fibrin(ogen), were shown to also confer platelet-neutrophil interactions through Mac-1. The immunoreceptor tyrosine-based activation motif (ITAM) receptor GPVI, the main platelet receptor for collagen, aids in local and systemic immune responses by promoting platelet-neutrophil aggregate formation and neutrophil recruitment in a model of gram-negative, pneumonia-driven sepsis (19). A recent study found that septic patients requiring intensive care treatment displayed acquired dysfunctions in GPVI-mediated platelet activation (20).

Interactions Through Secreted Molecules and Cytokines

While not directly mediating platelet-neutrophil interactions, numerous receptors are implicated in activation of platelets, which subsequently promote neutrophil binding and propagation of activation. These receptors include TLRs like TLR4, which promote both neutrophil activation and neutrophil extracellular trap (NET) formation in endotoxemia and gram-negative sepsis (8), complement receptors such as C3aR, which enhance thromboinflammation and neutrophil-mediated damage in myocardial infarction (21), and eicosanoid receptors (**Figure 1B**). Regarding the latter, a seminal study recently described the shuttling of neutrophil-derived arachidonic acid into platelets in a P-selectin-dependent manner, which was processed to thromboxane A2 (TXA$_2$) by activated platelets (22). Subsequent TXA$_2$ secretion induced endothelial cell activation, ICAM-1 expression and promoted neutrophil recruitment to sites of inflammation. Consequently, targeting of platelet activation through blockade of soluble agonist receptors such as the adenosine-diphosphate (ADP) receptor P2Y$_{12}$ reduced platelet-neutrophil interaction and attenuated thromboinflammation in endotoxemia (23, 24).

Platelet secretion of several soluble mediators affects neutrophil functions under steady-state conditions and inflammation alike: Platelet-derived heterodimers consisting of platelet factor 4 (PF4) and CCL5/RANTES (regulated on activation, normal T cell expressed and secreted) stored in alpha granules (2, 25) promote neutrophil extravasation in acute lung injury (26) and are known to modulate neutrophil function in sterile inflammation (27). Similarly, soluble P-selectin dimers shed by activated platelets promote leukocyte adhesion and NET formation under inflammatory conditions in mice (28). Further, High mobility group box 1 (HMGB1) secreted by activated platelets enhances neutrophil recruitment by activating Receptor for advanced glycation endproducts (RAGE) on neutrophils

FIGURE 1 | Direct and indirect platelet-neutrophil interactions. **(A)** Overview of neutrophil (left, petrol) and platelet receptor pairs (right, yellow) that directly engage and promote reciprocal activation of either cell type. **(B)** Overview of soluble agonists secreted by platelets promoting neutrophil activation (left panel) and vice versa (right panel). CTSG, cathepsin G; ELANE, neutrophil elastase; EV, extracellular vesicles; HMGB1, High mobility group box 1; ROS, reactive oxygen species; TXA$_2$, thromboxane A$_2$. Created with BioRender.com.

(29). Similarly, serotonin stored in platelet dense granules promotes neutrophil CD11b expression, oxidative bursts and degranulation in mice and men (30). Vice-versa, neutrophil-derived agonists such as cathelicidins (LL37 in humans, CRAMP in mice) can activate platelets and thereby propagate thrombus formation, as shown in mouse models of arterial thrombosis and histopathological analyses of human thrombi (31). Further, enzymes such as ELANE and CTSG as well as ROS released by

neutrophils promote platelet activation (32) through cleavage of protease-activated receptors (PAR), as recently shown for CTSG and PAR4 (33). This reciprocal influence of both cell types is also observed at the level of extracellular vesicles (EVs): While platelet-derived EVs promote NET formation in septic shock and dengue virus infection (34, 35) and are able to shuttle regulatory RNA to neutrophils (36), neutrophil-derived EVs transfer metabolites to platelets and thereby promote reciprocal activation (22).

In summary, platelets and neutrophils interact and co-operate through a vast variety of receptors, soluble agonists and even EV-mediated shuttling of effector molecules. Since some of the secreted agonists mentioned above are especially released after physical interaction of both cell types, direct binding and subsequent agonist release may promote reciprocal (hyper)activation at both the local and the systemic level (5, 37).

RECIPROCAL PLATELET AND NEUTROPHIL ACTIVATION DRIVES IMMUNOTHROMBOSIS

Reciprocal binding of platelets and neutrophils leads to phenotypical changes in both cell types. Classical neutrophil responses upon platelet binding include conformational changes of adhesion and activation receptors such as CD11b and CD177, but also shedding of surface molecules like L-selectin (CD62L) (1). In neutrophils, MAPK- and Syk-mediated signaling upon platelet interaction mediates production and release of the neutrophil chemoattractant CXCL8/interleukin-8 (IL-8) and beta-integrin activation, enhancing neutrophil recruitment and adhesion to ICAM-1-positive endothelial cells (38).

Functionally, platelet binding can boost phagocytic capacity and bacterial clearance exerted by neutrophils, as well as production and ROS secretion under inflammatory conditions (11, 39). Immuno-responsive platelets also collect and bundle pathogens, aiding in local control of infection (40). Consequently, thrombocytopenia is associated with reduced neutrophil activation and impaired bacterial clearance (41). One of the most striking features of platelet-neutrophil interactions is the ability of platelets to trigger neutrophil extracellular trap (NET) formation, a specific death program by which neutrophils release nuclear DNA covered with histones and neutrophil effector enzymes into blood and the interstitium (41). This process, which is triggered by P-selectin/PSGL-1 interactions (42) and likely represents an ancient defense mechanism to prevent spreading of a variety of pathogens including bacteria, viruses, and fungi (8, 35, 43–47), enables neutrophils to "ensnare" circulating microbes and facilitates clearance by phagocytes (8, 41, 48). Consequently, pharmacological or genetic ablation of NET formation or cleavage of NETs by exogenous DNases aggravates systemic bacterial load and is associated with worsening outcome in models of bacterial infection (8, 46, 48). The complex molecular mechanisms underlying NET formation and resolution are discussed elsewhere (37, 49, 50).

In addition to facilitating pathogen capture, NET formation also promotes coagulation through a plethora of mechanisms like histone-mediated platelet activation, cleavage of tissue factor pathway inhibitor (TFPI) and thrombomodulin by effector enzymes like ELANE and CTSG, and direct binding of vWF and factor XII (46, 49, 51–53). *In vivo* live imaging studies of liver sinusoids have shown increased thrombin turnover locally associated with NETs, suggesting a direct stimulation of plasmatic coagulation through NETosis (54). However, only isolated DNA and histones, but not intact NETs, were shown to drive activation of the contact pathway and thrombin generation *in vitro* (55). Mechanistically, neutralization of negative charges of supercoiled DNA through histone/nucleosome binding is thought to underly the lack of procoagulant potential observed in isolated NETs (55).

The pathophysiological process that underlies inflammation-driven NETosis, recruitment of proinflammatory monocytes, platelet activation and increased coagulation with deposition of fibrin(ogen) is termed immunothrombosis. Immunothrombosis is considered a protective host defense mechanism designated to prevent microbial spreading (49). While enhancing pathogen capture during infection, dysregulated and excessive activation of immunothrombosis—termed thromboinflammation—can promote local ischemia and subsequent collateral organ damage. In addition, thromboinflammation has been attributed to remote organ injury, aggravation of systemic inflammation and disseminated intravascular coagulation (DIC) (37, 49, 56–59). In addition, excessive NET formation may be detrimental in some cases of bacterial infection: In models of infective endocarditis, NETs were shown to induce trapping and formation of platelet-bacteria aggregates on injured heart valves, thereby potentiating bacterial growth and vegetation expansion (60, 61).
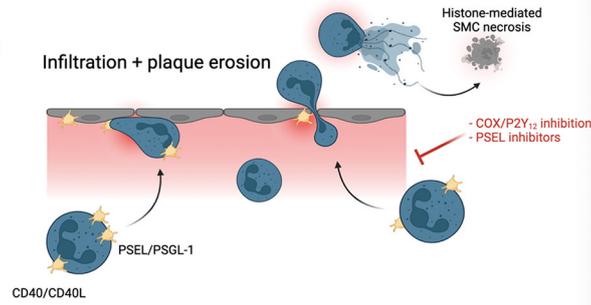
PLATELET-NEUTROPHIL INTERACTIONS IN CARDIOVASCULAR DISEASE

Chronic (Cardio)Vascular Inflammation

Cardiovascular events including myocardial infarction, ischemia-driven heart failure and stroke are all sequelae of atherosclerosis and remain the most frequent cause of death world-wide (62).
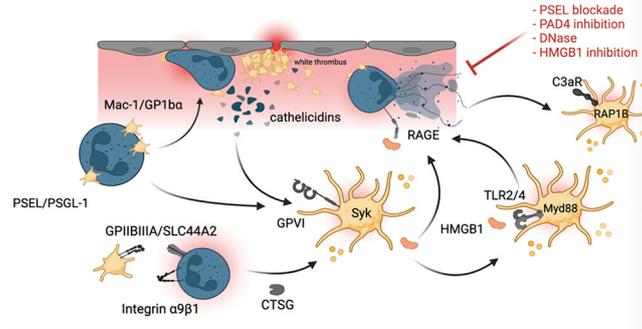
Since the early 2000s, evidence has emerged highlighting the role of platelet adhesion and subsequent leukocyte—and specifically neutrophil—recruitment in early atherogenesis (63) and the progression of atherosclerotic lesions (64, 65). This evidence is supported by more recent data that implicate a general role for platelets in atheroprogression through recruitment of other leukocyte subsets—namely monocytes and eosinophils (66, 67). Both neutrophilia and thrombocytosis as well as increased platelet-neutrophil aggregates (PNA) are observed in patients with chronic cardiovascular conditions such as peripheral (PAD) (68) and coronary artery disease (CAD) (69–71). Local recruitment of circulating neutrophils to atherosclerotic plaques is enhanced by platelets binding to inflamed endothelium (**Figure 2A**) (14, 72). Subsequent PNA formation has been shown to depend on P-selectin/PSGL-1 interaction, and elevated soluble P-selectin is observed in patients with cardiovascular disease and positively correlates with the risk of major adverse cardiovascular events (MACE) (73–75). In

FIGURE 2 | Continued
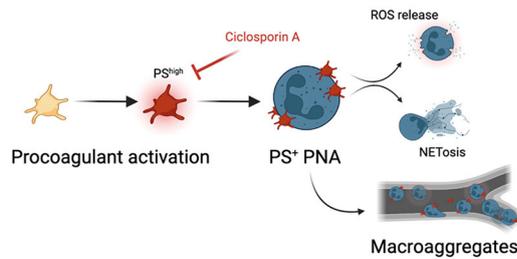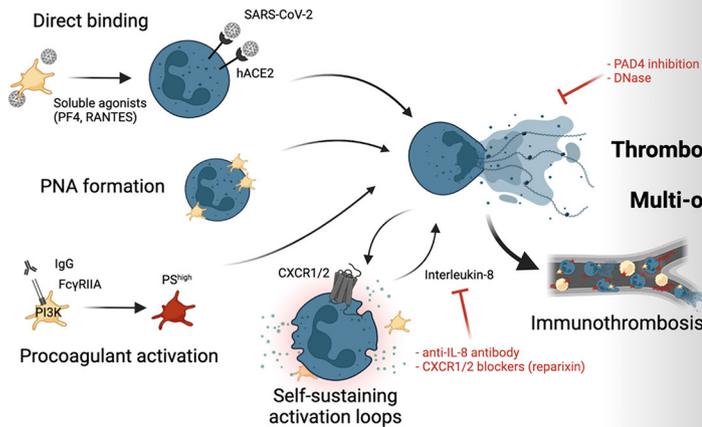
addition, CD40-CD40L interactions of platelets and neutrophils and other leukocyte subsets exacerbates atherosclerosis, while CD40 deficiency was associated with reduced atheroprogression (14). Enhanced adhesion of neutrophils to sites with high atherosclerotic plaque burden as well as neutrophil recruitment into the plaque are associated with plaque instability, erosion, and rupture (76–78). Activated neutrophils recruited into the plaque can exacerbate chronic vascular inflammation, specifically atherogenesis and atheroprogression, by NET formation, promoting a proinflammatory macrophage phenotype and inducing histone H4-mediated lytic cell death of smooth muscle cells inside the plaque (79, 80). Targeting endovascular platelet-neutrophil interactions before subsequent neutrophil adhesion and plaque recruitment ensue therefore provides therapeutic promise. The effects of platelet-neutrophil interactions in other entities of chronic cardiovascular inflammation, such as abdominal aortic aneurysms, are reviewed elsewhere (81, 82).

Thrombosis

As a consequence of atheroprogression, plaque erosion and—ultimately—plaque rupture, thrombogenic material including vWF, extracellular matrix proteins and cellular debris released by the necrotic core is exposed to circulating blood cells (76). Sensing of PAMPs and extracellular matrix proteins swiftly leads to the recruitment of platelets *via* GPIbα-vWF and GPVI–collagen interactions (2), which start to form a cellular clot called the "white thrombus" (83). These highly activated platelets recruit leukocytes in a Mac-1/GPIbα- and PSGL-1/P-selectin-dependent manner, promoting fibrin(ogen) deposition and enhancing clot stability (10, 84). Notably, the recruitment cascade appears to be model-dependent, and leukocyte recruitment to laser-induced arterial injury was shown to depend on LFA-1/ICAM-1 neutrophil-endothelium interactions, but not solely on platelets (85). Within this prothrombotic microenvironment, a self-sustaining loop of reciprocal platelet and neutrophil activation is initiated (**Figure 2B**): Activated neutrophils secrete cathelicidins (LL-37/CRAMP), which promote further platelet activation and aggregation through the collagen receptor GPVI and its downstream signaling cascades, including tyrosine kinase Syk (31). Next, platelets secrete HMGB1, which self-stimulates platelets *via* the TLR4-Myd88 axis and promotes NET formation through RAGE on neutrophils, thereby sustaining platelet activation and clot stability (29, 39). Platelet-neutrophil crosstalk in arterial thrombosis is enhanced by two additional mechanisms: A recent study revealed that neutrophilic α9β1 mediates platelet activation and arterial thrombus formation. Mechanistically, α9β1-promoted platelet activation reciprocally enhances NET

formation and secretion of CTSG, which in turn sustains arterial neutrophil recruitment (86, 87). Of note, blockade of platelet-induced NETosis through pharmacological or genetic ablation of Protein arginine deiminase 4 (PAD4) attenuates arterial thrombus formation (78, 88). Following neutrophil activation, excreted NETs promote platelet activation and aggregation through complement C3 activation and subsequent platelet signaling through the anaphylatoxin receptor C3aR and downstream RAP1B (21, 89). Consequently, C3aR deficiency reduces platelet activation and attenuates arterial thrombosis (90). The translational relevance of these observations in mouse models of arterial thrombosis are underlined by the presence of LL-37 and TF-positive NETs in coronary artery thrombi from patients with acute myocardial infarction and a marked NET signature in thrombi from patients suffering from coronary stent thromboses (31, 91–93).

In venous thrombosis, endothelial inflammatory responses and ICAM-1/VECAM expression promote swift recruitment of platelet and both monocytes and neutrophils alike and the formation of PNAs and platelet-monocyte aggregates (PMAs) (37, 94). The underlying molecular mechanisms are reminiscent of arterial thrombosis and involve PSEL/PSGL-1 interactions, HMGB1-induced platelet and neutrophil activation through TLR2/4 and RAGE, respectively, and propagation of immunothrombosis through NET formation (29, 37). A novel interaction between activated GPIIBIIIA on platelets and the choline transporter SLC44A2 on neutrophils was recently observed under low shear rate. This interaction enhanced NET formation and thereby propagated deep vein thrombosis (95). Supporting the role of neutrophils and platelets in venous thrombosis, both genetic ablation of P-selectin, blockade of HMGB1 receptors and therapeutic targeting of NETs through exogenous DNases or genetic ablation of PAD4 resolved thrombus formation (52, 94, 96).

Ischemic Stroke and Ischemia-Reperfusion Injury

Ischemic stroke, triggered by either cardiac embolism or carotid plaque rupture, is characterized by systemic increases in circulating neutrophils and formation of platelet-neutrophil aggregates, with the extent of neutrophilia correlating with infarction size and patient prognosis (97, 98). In a pivotal study, Denorme et al. (99) described a hazardous interplay between neutrophils and a hyperactivated subtype of platelets, so-called procoagulant platelets, in mice. This platelet population, which is induced upon combined stimulation of either strong soluble agonists or high mechanical shear stress, is hallmarked by high

levels of phosphatidylserine (PS) exposure, microvesiculation and striking morphological changes, namely platelet ballooning (100–103). In a model of ischemia-reperfusion injury (IRI) of the brain, procoagulant platelets were induced systemically, subsequently promoted recruitment of neutrophils across the blood-brain-barrier (BBB), leading to intracerebral ROS release, NETosis and exacerbated brain damage (**Figure 2C**). Targeting procoagulant platelet activation through genetic ablation or by pharmacological blockade of PS attenuated cerebral damage and enhanced neurological outcomes (99, 104, 105).

Platelet-neutrophil interplay also affects IRI in other organs, such as heart, liver and kidney, as described elsewhere (106–109). Of note, reperfusion injury in the lung is attributed to platelet-neutrophil interplay: While platelet-dependent NET formation was observed in experimental models and clinical samples of lung transplant dysfunction (110), Yuan et al. found that gut ischemia induced the formation of PS$^+$ platelet-neutrophil aggregates in the mesenteric vasculature, which subsequently led to uncontrolled NETosis and the formation of platelet-neutrophil macroaggregates occluding the lung vasculature (111). Targeting either platelet-neutrophil interactions or platelet-induced NETosis alleviated intravascular thrombosis and enhanced outcomes, suggesting PNA and NET formation as potent clinical targets.

COVID-19-RELATED IMMUNOTHROMBOSIS

The COVID-19 pandemic has impacted global human life, and an unprecedented research effort has been initiated to understand and counter this viral infection caused by SARS-CoV-2. Severe cases are defined by hyperinflammation reminiscent of cytokine release syndromes (112). Interestingly, patients with cardiovascular comorbidities are at high risk of developing severe COVID-19, which is associated with an increased incidence of venous and arterial thrombotic complications like pulmonary embolism and myocardial infarction (113–115). Indeed, a systemic procoagulant state correlating with disease severity was identified in COVID-19 patients (57). As outlined above, thrombosis in inflammatory disease has been linked to intravascular interplay of platelet and innate immune cells in the framework of immunothrombosis (46, 116). Along these lines, thrombocytosis and neutrophilia were described in severe cases of SARS-CoV-2 infection, and both cell types showed unique activation patterns compared to healthy controls and other viral infections (37, 49, 56–59).

Mechanistically, this immunothrombotic state seems to at least in part be mediated by neutrophil-platelet interplay: *In vitro*, addition of platelets derived from severe COVID-19 patients to healthy neutrophils induced activation and NETosis (57). This seems to be triggered by direct interactions, but also by the release of soluble mediators like RANTES and PF4 by platelets, known to be involved in NET formation (**Figure 2D**) (117). Platelet transcriptomics revealed upregulated protein ubiquitination, antigen presentation, and mitochondrial dysfunction in COVID-19 patients (118). This coincided with increased MAPK signaling and higher numbers of circulating platelet-leukocyte aggregates (PLA), including PNAs (118). In summary, platelets are primed to interact and trigger neutrophil activation in SARS-CoV-2 infection, which in turn leads to neutrophil activation, granule release, and NETosis, triggering immunothrombotic dysregulation. Preceding platelet activation seems to be induced by three key events: An important mechanism is vascular inflammation and endotheliopathy induced by this betacoronavirus, which leads to endothelial disruption and platelet activation in the vasculature (59, 116, 119). Second, direct association and uptake of SARS-CoV-2 viral particles by platelets is observed, which triggers hyperactivation (118, 120). Third, a research group identified increased procoagulant potential indicated by PS exposure and intracellular calcium elevation, induced by autoantibodies and driven by the phosphoinositid-3-kinase (PI3K)/protein kinase B (AKT) signaling pathway (121, 122). Interestingly, procoagulant function was induced by Fcγ receptor IIA binding of plasmatic IgG antibody fractions of COVID-19 patients (121). Procoagulant platelets are also known to preferentially interact with neutrophils, contributing to thromboinflammation in experimental stroke, possibly contributing to the increase in thrombotic events in patients with severe courses (99). In conclusion, platelet-neutrophil interplay is crucial in shaping the prothrombotic phenotype observed in COVID-19 and might offer a path to therapeutic intervention.

THERAPEUTIC TARGETING OF PLATELET-NEUTROPHIL INTERACTIONS

As outlined above, targeting platelet-neutrophil interplay might be a valuable tool to fight cardiovascular disease, in particular acute thromboinflammation. One promising approach are the P-selectin antibodies inclacumab and crizanlizumab that block interaction of platelet and endothelial P-Selectin with neutrophilic PSGL-1. *In vitro* experiments confirmed that inclacumab inhibits PLA formation (123). A landmark study, SELECT-ACS, showed that inclacumab at a dosing of 20 mg/kg significantly reduced myocardial damage assessed by CK-MB levels after percutaneous coronary intervention in patients with non-ST-segment elevation myocardial infarction (124, 125). In contrast, the SELECT-CABG Trial showed no effect of inclacumab on bypass graft failure after coronary bypass (126). Crizanlizumab, also blocking PSGL-1/P-selectin interaction, was shown to be safe and effective at preventing vasoocclusive crises mediated by platelet-neutrophil interactions in sickle cell disease (SUSTAIN study) (127). Importantly, receiving anti-P-selectin antibodies was not associated with increased infection or bleeding rates, while other adverse events like diarrhea and chest pain exceeded those observed in placebo-treated patients (124–127). Further studies are needed to assess the potential of these antibodies in acute and chronic coronary syndromes. Another study targeting PLAs in pneumonia with Ticagrelor (XANTHIPPE) (128) revealed that ticagrelor reduced the fraction of platelet-bound leukocytes and IL-6 levels, and also decreased oxygen demand compared to placebo-treated

patients without affecting the incidence of adverse events. Ticagrelor has been shown to be superior to clopidogrel in preventing death from vascular causes, myocardial infarction, or stroke, but the mechanisms are not fully understood and might involve reductions in circulating PLAs (129). In experimental settings, additional therapeutic approaches that focus on interference with other platelet receptors like GPIbα have been shown to decrease neutrophil recruitment into inflamed tissue (130).

Targeting leukocyte integrins has proven a valuable therapeutic approach for refractory chronic inflammatory diseases (131, 132), and therapeutic interference with neutrophil Mac-1 attenuates both inflammation and thrombosis in experimental settings (10, 133, 134). As outlined above, NETosis is a common effector mechanism of neutrophils triggered by platelets and holds an important role in mediating intravascular thrombosis and clot formation. Therefore, pharmacological inhibition and degradation of NETs represents a promising approach in a range of acute and chronic inflammatory conditions. The anticoagulant heparin, used widely in acute thrombotic events, disrupts NETs and neutralizes histones, which contributes to its clinical effect (135, 136). In cystic fibrosis patients, treatment with inhaled NET degrading rhDNase1, known as dornase alfa, was associated with improved oxygenation and decreased DNA:MPO complexes in BALF (137). Ongoing studies are addressing its effects in COVID-19 (NCT04409925) and ischemic stroke (NCT04785066).

PF4 and CCL5/RANTES are important platelet chemokines shaping innate intravascular immune responses. The antiretroviral drug Maraviroc targets the CCL5 receptor CCR5, and was developed as an entry inhibitor against HIV, since CCR5 is a co-receptor for viral entry (138). In addition to its antiviral properties, Maraviroc was found to reduce PLAs and markers of atherosclerosis like carotid intima-media thickness in HIV patients (139). This was also confirmed in an experimental mouse model of atherosclerosis (140). A naturally occurring loss-of-function mutation in CCR5 was also found to be protective after stroke and traumatic brain injury, however, these effects seem to also directly influence neurons beyond affecting platelet-leukocyte interplay (141). Studies addressing the efficacy of Maraviroc in stroke have been initiated (NCT04789616).

CONCLUSIONS

Recent evidence has highlighted novel interaction pathways between neutrophils and platelets that crucially effect acute thromboinflammation and chronic vascular disease alike. Further translational studies as well as clinical trials will help to pin down the most effective therapeutic strategies depending on disease manifestation and patient characteristics.

AUTHOR CONTRIBUTIONS

RK, RE, and LN reviewed the literature and wrote the initial draft. RK and JE prepared the figures. All authors reviewed and approved the final version of the manuscript.

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