Platelet–Neutrophil Crosstalk in Atherothrombosis

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Abstract

Atherothrombosis is a frequent cause of cardiovascular mortality. It is mostly triggered by plaque rupture and exposure of the thrombogenic subendothelial matrix, which initiates platelet aggregation and clot formation. Current antithrombotic strategies, however, target both thrombosis and physiological hemostasis and thereby increase bleeding risk. Thus, there is an unmet clinical need for optimized therapies. Neutrophil activation and consecutive interactions of neutrophils and platelets contribute mechanistically to thromboinflammation and arterial thrombosis, and thus present a potential therapeutic target. Platelet–neutrophil interactions are mediated through adhesion molecules such as P-selectin and P-selectin glycoprotein ligand 1 as well as glycoprotein Ib and macrophage-1 antigen, which mediate physical cell interactions and intracellular signaling. Release of soluble mediators as well as direct signaling between platelets and neutrophils lead to their reciprocal activation and neutrophil release of extracellular traps, scaffolds of condensed chromatin that play a prothrombotic role in atherothrombosis. In this article, we review the role of neutrophils and neutrophil-derived prothrombotic molecules in platelet activation and atherothrombosis, and highlight potential therapeutic targets.

Keywords
- arterial thrombosis
- atherothrombosis
- thrombo-inflammation
- platelet–leukocyte interaction

Introduction

Cardiovascular diseases are the most common cause of death in developed countries. About 80% of cardiovascular deaths are caused by myocardial infarction and stroke following rupture of an atherosclerotic plaque and subsequent arterial occlusion. While an increased understanding of the mechanisms underlying these conditions has resulted in the development of novel antithrombotic strategies that reduced cardiovascular death, for example, thienopyridines, mortality has remained on a significant level. The relevance of these conditions is fueled by the increasing prevalence of risk factors for atherosclerosis in developing countries, such as diabetes and adipositas. Therefore, there is an unmet clinical and socioeconomical need for new treatment strategies. This article highlights recent findings on novel therapeutic targets in atherothrombosis with focus on neutrophil-derived activators of platelets and thrombus formation.

Established Pathophysiological Aspects and Current Therapeutic Strategies

In atherosclerosis, sustained inflammation drives formation of atheromatous plaques. The latter are typically composed of lipids, leukocytes, smooth muscle cells, and covered by a fibrous cap.[1–4] Eventually plaque rupture triggers arterial thrombosis, which manifests clinically as myocardial infarction, ischemic stroke, or limb ischemia in patients with peripheral artery disease. Mechanistically, highly thrombogenic plaque content

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becomes exposed to the blood stream and components such as collagen and von Willebrand factor (vWF) then mediate platelet adhesion and activation (►**Fig. 1A**).[5–7] Activated platelets release adenosine diphosphate, serotonin, and thromboxane A2 (TxA2), which amplify activation and induce platelet aggregate formation. Additionally, P-selectin is highly upregulated on the surface membrane of platelets to mediate interactions with leukocytes and endothelial cells.[8] Simultaneously, plaque-released tissue factor activates the coagulation cascade and triggers thrombin generation and subsequent conversion of fibrinogen into insoluble fibrin.[9,10] Thrombin stabilizes the forming clot by amplifying blood coagulation and by activating platelets which further propagates coagulation.[11]

In addition to urgent mechanical revascularization of the obstructed vessel to restore blood flow, current pharmacological strategies for the treatment of acute atherothrombotic events include platelet inhibition and anticoagulation (►**Fig. 1A**). Current guidelines recommend intravenous administration of unfractionated heparin in the acute setting and platelet inhibition with aspirin, P2Y12 inhibitors, and, as bailout therapy, glycoprotein (GP) IIb/IIIa inhibitors.[12,13] Yet, all antiplatelet drugs as well as anticoagulants are associated with an increased risk of bleeding, which underlines the necessity for further research into antithrombotic strategies.[14]

Neutrophil Activation and Platelet–Neutrophil Interactions in Atherothrombosis

Neutrophils play a role in different pathophysiological processes of atherosclerosis.[15–19] They support the development of early atherosclerotic lesions,[15] but have also been associated with features of plaque rupture.[16] Upon plaque rupture, together with platelets, neutrophils rapidly accumulate at sites of injury, and can induce and amplify platelet activation and blood coagulation[20–25] (►**Fig. 1A**). Notably, neutrophils represent the most abundant leukocyte subset also in arterial thrombi of human patients with myocardial infarction.[26] Neutrophils are stimulated by inflammatory cytokines such as interleukin-1β or tumor necrosis factor-α, which are elevated in plasma in atherosclerosis.[1,27] Activated neutrophils express adhesion molecules on their surface, which promote binding to platelets and the endothelium.[24] Vice versa, also activated platelets express adhesion molecules on their surface membrane, such as P-selectin, which mediate mechanical interactions with neutrophils.[28] Indeed, Sreeramkumar et al showed that neutrophils scan for activated platelets and foster their recruitment to inflamed or injured vessel wall thereby initiating and promoting inflammation.[29] It is therefore not surprising that circulating platelet–neutrophil aggregates represent an independent predictor of atherothrombotic events.[24,30–32] However, it is unclear whether these aggregates are a result of systemic inflammation during development of atherosclerotic lesions.

On the molecular level, platelet–neutrophil interactions lead to reciprocal activation of either cell type (►**Fig. 1B**). Several studies have described how platelets affect leukocyte functions and recruitment.[33,34] Platelets have a repertoire of cytokines and chemokines such as CCL5 (RANTES) and CXCL7, that are stored in their granules (mainly α-granules) and are able not only to recruit but also to activate neutrophils.[35,36] Additionally, platelet-derived microparticles and extracellular vesicles can transport and provide neutrophil activating molecules.[37] Moreover, fragments of dying platelets foster neutrophil aggregation after ischemia reperfusion injury.[38] Antiplatelet therapy in acute atherothrombosis effectively inhibits platelet aggregation and inhibiting platelet activation reduces the release of neutrophil-activating substances from α-granules.[8] In a mouse model of acute lung injury, where platelet–neutrophil interactions play a key mechanistic role, platelet depletion as well as aspirin therapy reduced neutrophil infiltration and provided protective effects.[39–41] This aspect was recently confirmed in human models of acute respiratory distress syndrome.[42] Thus, platelet–neutrophils interactions play a role in the wider context of thromboinflammation, and their inhibition could represent an interesting concept in these conditions.

Less is known about the effects of neutrophils on platelets as a result of aforementioned interactions. Activated neutrophils can combat microbes by releasing antimicrobial molecules (by exocytosis), phagocytosis of microbes (which are destroyed intracellularly by reactive oxygen species [ROS] and antimicrobial proteins), and by formation of neutrophils extracellular traps (NETs).[43] These mechanisms could differentially influence different aspects of atherothrombosis including platelet function. Specific targeting of leukocyte-derived platelet activating agents could represent an alternative approach in atherothrombosis and may have reduced hemorrhagic side effects.

Platelet Activation through Physical Interaction with Neutrophils

To prevent or reduce platelet–neutrophil interactions and their consequences, targeting the adhesion molecules that mediate the physical interactions between the two cells would be the most straightforward approach. A therapeutic challenge is the fact that most adhesion molecules do not have a single respective ligand but share several counter-receptors including those expressed on other cell types.

P-Selectin and P-Selectin Glycoprotein Ligand 1

Binding of platelet P-selectin and neutrophil P-selectin glycoprotein ligand 1 (PSGL-1) might constitute the most important physical interaction between platelets and neutrophils.[44] P-selectin is stored in platelet α-granules with little surface expression under resting conditions and is highly upregulated on the surface membrane when platelets become activated.[8]

While PSGL-1-induced activation of neutrophils via downstream signaling of tyrosine kinases and consecutive integrin activation is well described,[45,46] less is known about platelet activation after binding of P-selectin. Recombinant PSGL-1 induced integrin activation on platelets in a P-selectin-dependent manner and increased platelet aggregation-driven large artery thrombosis in vivo.[47] Further, disruption

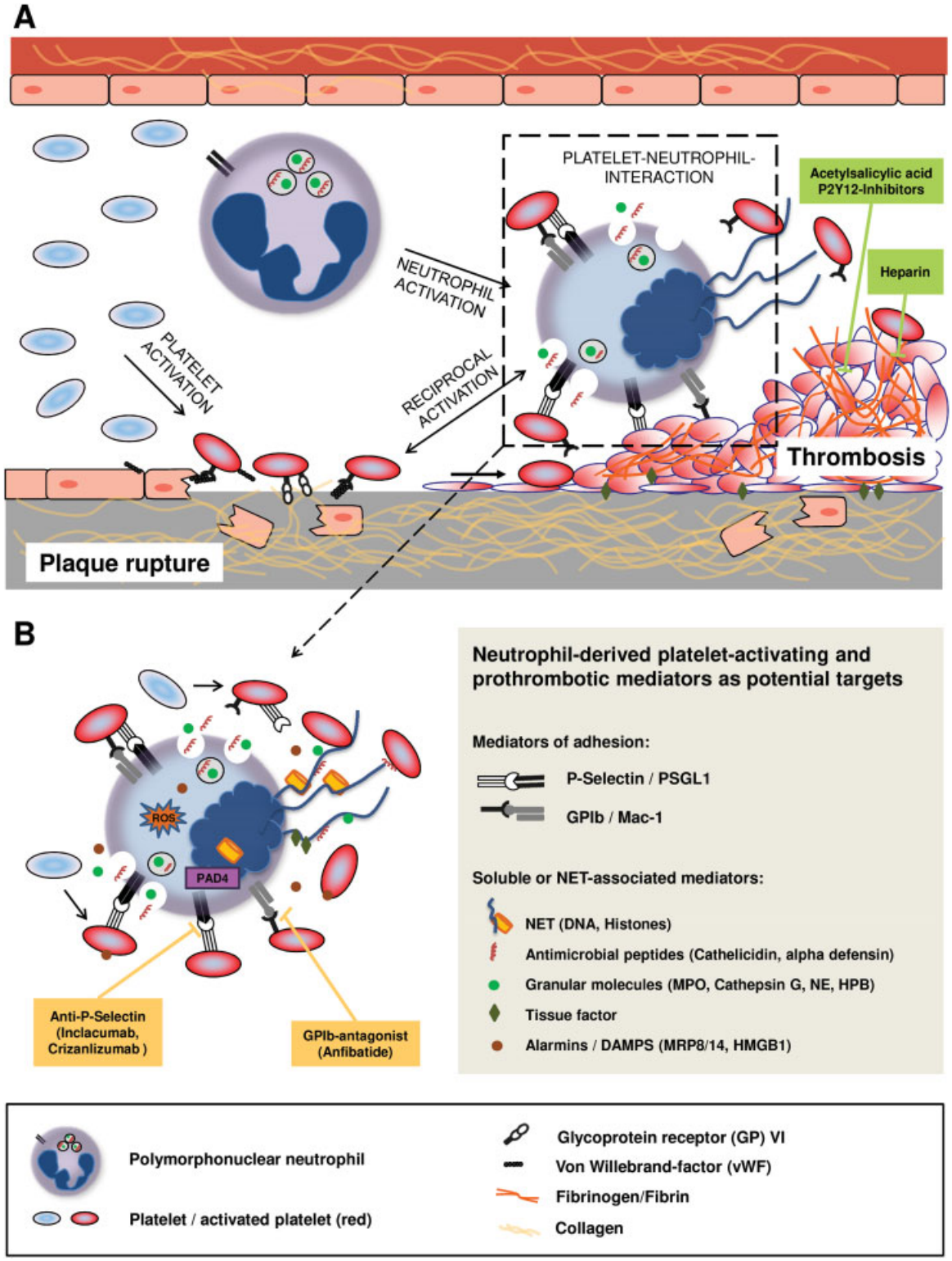

Fig. 1 (**A**) Upon rupture of an atherosclerotic plaque, thrombogenic proteins of the subendothelial matrix are exposed to the blood stream and initiate thrombus formation. Together with platelets, neutrophils rapidly accumulate at sites of injury, become activated, and can induce and amplify platelet activation and blood coagulation. Current pharmacological approaches include anticoagulation and platelet inhibition (green boxes). (**B**) Upon activation, platelets and neutrophils interact through adhesion molecules and soluble mediators and thereby reciprocally amplify activatory signals. The illustration depicts adhesion molecules and neutrophil-derived mediators that play a role in platelet activation and atherothrombosis and constitute potential therapeutic targets (gray box). Targets that have already been tested in first clinical trials are highlighted (orange boxes). DAMP, damage-associated molecular pattern; GP1b, glycoprotein 1b; HMGB1, high-mobility group box 1; HPB, heparin-binding protein; MPO, myeloperoxidase; MRP8/14, myeloid-related protein 8 and 14 (calprotectin, S100A8/A9); NE, neutrophil elastase; NET, neutrophil extracellular traps; PSGL-1, P-selectin glycoprotein ligand 1; vWF, von Willebrand-factor.

of P-selectin-PSGL-1-binding reduced fibrin incorporation upon laser injury-induced thrombosis in arterioles.[48,49] First clinical trials targeting platelet–leukocyte interactions using monoclonal antibodies against P-selectin have been initiated and proven safe.[50] Crizanlizumab lowered the rate of pain crisis in patients with sickle cell disease[51] and the monoclonal P-selectin antibody inclacumab reduced myocardial damage after percutaneous coronary intervention in patients with non-ST-segment elevation myocardial infarction.[52,53] In mice, deficiency in PSGL-1 improved outcome in thromboinflammation models such as acute lung injury and ischemic stroke.[29] Targeting P-selectin seems promising also in atherothrombosis as treatment of mice with anti-P-selectin antibodies has been shown to directly reduce thrombus size in a mouse model of ferric chloride-induced arterial thrombosis.[54]

GPIb and Macrophage-1 Antigen

Platelet GPIb receptor is constitutively active for ligand binding and represents an important mediator of platelet–neutrophil interactions. Interestingly, platelet-derived protein disulfide isomerase (PDI) regulates the binding function of GPIbα by reducing its disulfide bonds. Consequently, inhibition or genetic absence of PDI abrogated platelet–neutrophil interactions and inhibited vascular occlusion under thromboinflammatory conditions.[55] Among various potential partners, GPIb binds to the β-2 integrin receptor macrophage-1 antigen (Mac-1) on neutrophils to form platelet–neutrophil aggregates.[7,24,29] Mac-1 deficient mice were protected from thrombosis after glomerular injury[56] and show delayed thrombosis after carotid artery and cremaster microvascular injury.[57] These effects could also be observed by targeting Mac1–GPIb interaction with antibodies and the small-molecule inhibitor glucosamine. Importantly, no effects on hemostasis parameters were observed, making Mac-1 an interesting therapeutic target.[57] Besides binding to GPIb, neutrophil Mac-1 has also been described to bind platelet GPIIb/IIIa-integrin via bridging of soluble fibrinogen[7,58] and might contribute to platelet activation through outside-in signaling.[59] Further, microparticles derived from activated neutrophils express an active form of Mac-1 that can induce P-selectin surface expression and integrin activation in platelets via GPIb-mediated Akt phosphorylation.[60]

Direct targeting of GPIb could represent another perspective for treatment of atherothrombosis. Such approach would not only affect platelet–neutrophil crosstalk but simultaneously inhibit GPIb interaction with vWF, which is known to contribute to atherothrombotic events.[5,6] Several clinical trials with therapeutics targeting the GPIb-vWF axis have been made.[61] The humanized anti-vWF bivalent nanobody caplacizumab (ALX-0081) has already undergone first clinical tests with promising results.[62,63] Similarly, the GPIb antagonist anfibatide reduced thrombus formation in mice without prolonging bleeding time[64] and safety and efficacy trials in humans are ongoing (ClinicalTrials.gov Identifier: NCT01585259, NCT02495012).

Platelet–neutrophil interaction is promoted and stabilized by several other ligand–receptor interactions, such as GPVI and extracellular matrix metalloproteinase inducer (EMMPRIN) or intercellular adhesion molecule 2 (ICAM-2) and lymphocyte function-associated antigen 1 (LFA-1).[7,24,65] While EMMPRIN–GPVI has been investigated in the context of platelet–monocyte interactions,[65] little is known about its role in platelet–neutrophil crosstalk. Binding of platelet ICAM-2 with neutrophil LFA-1 can mediate platelet–neutrophil adhesion under flow,[66] however, the physiological relevance for platelet and neutrophil function is not yet understood.

Platelet Activation through Soluble Neutrophil-Derived Mediators

Neutrophil granules store a broad repertoire of molecules, which upon activation are released or translocated to the surface.[43] In the context of atherothrombosis, these molecules will come in contact with platelets and coagulation factors and might differentially affect thrombus formation.

Neutrophil-derived peptides such human α-defensins (HNP 1–3) or heparin-binding protein have been shown to affect fibrinogen binding to platelets resulting in platelet aggregation and activation of the coagulation system.[67–70] In contrast, myeloperoxidase (MPO) has been described to induce weak activation of platelets without inducing aggregation, which primes platelets by potentiating agonist-induced platelet aggregation.[71,72] The neutrophil granular peptide cathepsin G has been shown to activate platelets in vitro[73] and arterial thrombosis in vivo,[67] which was explained by proteolysis of the coagulation suppressor tissue factor pathway inhibitor (TFPI)[20] and activation of factor XII (FXII).[74] Neutrophil elastase apart from cleaving TFPI also enhances coagulation by cleavage of another anticoagulant, antithrombin,[75] and degradation of proteoglycans in the arterial media, thereby exposing collagen.

Another abundant neutrophil-derived protein is S100A8/A9 (MRP8/14), which can also be secreted by platelets.[76] It increases neutrophil recruitment[77] as well as platelet activation and thrombosis in photochemical injury of the carotid artery in mice.[76] MRP8/14 is elevated in early steps of myocardial infarction and could again be a target to treat the acute atherothrombotic event as well as consecutive inflammation.[78]

Recently, we detected neutrophil-derived cathelicidins, a family of antimicrobial peptides, in thrombi from coronary arteries in acute myocardial infarction and demonstrated a functional role in platelet activation and arterial thrombosis which is in part mediated by reciprocal activation of neutrophils and platelets and by consecutive formation of NETs. Interestingly, these effects seem less dependent on classical cathelicidin receptors but rather are mediated by functional GPVI.[25] We are currently investigating whether other peptides with similar properties could play a role in atherothrombosis.

Upon interaction with platelets, neutrophils have been shown to release extracellular vesicles containing arachidonic acid which can be internalized into platelets and serve as a substrate for cyclooxygenase 1, an enzyme that synthesizes

TxA2.[79] TxA2 is strong paracrine amplifier of platelet aggregation and endothelial activation.[80]

NETs in Arterial Thrombosis

NET release (or NETosis) was first described by Brinkmann et al as an active form of cell death that releases a scaffold of condensed chromatin and antimicrobial proteins.[81] However, NET formation is clearly distinct to apoptosis and necrosis.[82] Neutrophils undergo histone citrullination by an enzyme called protein arginine deiminase 4 (PAD4).[83] In detail, PAD4 converts arginyl residues in histones (particularly H3 and H4) to citrulline which releases the ionic bonds that usually constrain nuclear deoxyribonucleic acid (DNA) to nucleosomes. This leads to dissociation of linking histones and heterochromatin from the nucleosome structure,[84,85] and eventually the nuclear chromatin network is released into the extracellular space and the surrounding tissue.[81,82] ROS and neutrophil granular enzymes significantly aid this process.[86] Several neutrophil activating agents triggering NET formation have been identified; however, differences in the kinetics may occur.[87] More rapid formation of NETs has been described when neutrophils are exposed to Gram-positive bacteria suggesting involvement of other so far not well-known mechanisms.[88]

While NETs are protective in infectious conditions, in recent years they have been shown to be involved in the pathology of various inflammatory diseases including atherothrombosis.[19,87,89] NETs have been detected in the carotid artery in apolipoprotein E-deficient mice on high-fat diet[90] and are clinically associated with coronary atherosclerosis.[91,92] Further, NETs have been found within arterial thrombi of mice and humans in acute myocardial infarction.[18,25,26,93] The majority of neutrophils in arterial thrombi stained positive for citrullinated histone 3,[25] a marker for neutrophil priming toward NET formation (NETosis),[83] indicating a highly activated state of neutrophils in arterial thrombosis. It has been shown that inhibition of the enzyme PAD4 (which is required for NET formation) with Cl-amidine, which abrogated NET formation, reduced atherosclerosis burden and arterial thrombosis in mice, and further limited injury in a model of myocardial infarction.[23,26] Genetic deficiency of PAD4 decreased deep vein thrombosis[22,94] and reduced acute thrombotic complications of intimal atherosclerotic lesions in mice.[95] It has been intensively studied how or which components of NETs are responsible for its prothrombotic effects.

Prothrombotic and Platelet Activating Properties of NETs

NETs are composed of a core DNA element, to which histones and neutrophil granular proteins (e.g., lactoferrin, cathepsins, cathelicidins, MPO, and neutrophil elastase) are attached.[43] NETs can serve as mechanical scaffolds for the entrapment and aggregation of platelets and erythrocytes in thrombosis, but additionally bind plasma proteins like fibrinogen, fibronectin, and vWF and thereby can stabilize clots.[22] Theoretically, however, NET components can separately participate in atherothrombosis and contribute to the activation of platelets, endothelial cells, or the coagulation system, and not least, different types of leukocytes. Negatively charged nucleic acids can activate the contact-dependent coagulation pathway in vitro, which surprisingly was not observed by entire NETs.[96] However, whether the DNA backbone itself contributes to atherothrombosis in vivo is not fully understood. Even though DNAse treatment did not attenuate arterial thrombus formation in healthy mice,[97] arterial thrombosis was impaired in mouse models of lupus and atherosclerosis, where animals showed generally more NET formation.[23,98] Histones have been attributed a key role in recruiting platelets and promoting their activation.[22] Histones have been described to induce platelet aggregation, P-selectin expression, phosphatidylserine exposure, and enhanced cell surface binding of FV/Va, which were in part mediated by Toll-like receptor 2 (TLR2) and TLR4.[99] Further, histones enhanced coagulation by inducing prothrombinase activity[99] (at least to some extent) by inhibition of thrombomodulin-dependent generation of the anticoagulant protein C[100] and induced the endothelial release of vWF.[101] Infusion of antihistone antibody (anti–H2A-H2B-DNA) leads to prolonged time to occlusion and lower thrombus stability upon injury of carotid arteries in wild-type mice.[20] In line with this, the presence of NETs was associated with an increased thrombus stability and reduced thrombus resolution in an ex vivo study of human thrombus material.[102]

Additionally to the NET core structure itself, neutrophil-derived molecules presented on NETs could amplify thrombosis involving mechanisms described above. However, NETs might also represent a scaffold for other mediators of thrombosis. Tissue factor represents a highly prothrombotic molecule associating with NETs in venous as well as in arterial thrombi.[93,103] Hematopoietic or intravascular tissue factor plays a critical role in deep vein thrombosis.[104] Though monocytes are likely the major source of intravascular tissue factor, macrovascular thrombosis is also driven by vessel wall-derived tissue factor.[105] Moreover, neutrophils themselves may deliver tissue factor to NETs and thereby stimulate thrombin generation and platelet activation.[106] Nonetheless, the main function of neutrophils and their NETs in coagulation-dependent thrombosis lies in their ability to propagate the thrombotic process.[107]

Another protein which is not predominantly released by neutrophils but interacting with NETs is high-mobility group box 1 (HMGB1). HMGB1 induces NET formation in vitro[108,109] and also contributes to NETs in arterial thrombi in vivo.[110] HMGB1 is a chromatin-binding nuclear protein that stabilizes nucleosomes and stimulates gene transcription.[111] When HMGB1 is released by dying cells or actively secreted by stressed cells, it acts as a damage-associated molecular pattern molecule and shows strong proinflammatory properties in several diseases.[112] HMGB1 has also been found in arterial thrombi of mice with platelets being the major source[110] and is associated with activated platelets in patients with coronary artery disease.[113] HMGB1 induced platelet activation via TLR4 and MyD88-dependent

signaling.[110] Mice deficient in platelet HMGB1 display decreased arterial thrombus formation with prolonged bleeding time but no changes in coagulation parameters.[110]

Taken together, NETs seem to play an important role in atherothrombosis, in part through their composition of nucleic acids and histones as their backbone, serving as scaffolds for cells and plasma proteins but also by their high potential to expose the aforementioned inflammatory and prothrombotic molecules. Therefore, targeting NET generation might represent a powerful tool to treat acute atherothrombosis.

Mutations in the hematopoietic lineage leading to clonal hematopoiesis (CH) represent an emerging field of research in the context of atherosclerosis and thrombosis, and seem to affect both neutrophil and platelet biology. Somatic mutations are common in blood cells and increase with age, that is, the frequency is $>10\%$ of people aged >70.[114] They result in loss of function in epigenetic modifiers (e.g., *DNMT3A* or *TET2*) or increased hematopoietic signaling (*JAK2*), driving the clonal expansion of hematopoietic stem cells in a process termed CH. Notably, carriers of CH not only exhibit an increased risk for hematologic malignancies but are also prone to develop atherosclerotic cardiovascular disease. Thus, CH represents an important nontraditional risk factor underlying myocardial infarction and stroke.[115] Importantly, in a population study of 10,893 individuals without a known myeloid disorder, JAK2V617F positive CH was associated with higher incidence of thrombosis.[116] In transgenic mice expressing *Jak2*^{V617F}, platelet reactivity to agonists was enhanced.[117] Further, neutrophils expressed higher levels of peptidylarginine deiminase 4, which catalyzes citrullination of histones, and displayed increased NET formation.[116] These findings suggest that both platelets and neutrophils are players in CH-associated thrombosis. Future work will have to determine in more detail the function and interactions of platelets and neutrophils in atherothrombosis in patients harboring CH-associated mutations.

Conclusion

Aspects of acute inflammation are intriguingly linked with atherothrombosis and may provide several therapeutic targets in the future. To date, bleeding represents the most relevant side effect of all current pharmacological treatments. Targeting of platelet–neutrophil crosstalk, neutrophil-derived soluble, or surface bound mediators seems to have less impact on hemostasis than established antithrombotic therapies. While anti-inflammatory therapeutic strategies can also be associated with side effects as shown for long-term treatment with canakinumab,[27] short-term inhibition of inflammation in acute thrombosis is likely to be less problematic from an immunologic perspective. Undoubtedly, new avenues of research in platelet biology and immunothrombosis are driving the identification of novel therapeutic targets and their translation into clinical practice.

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Conflict of Interest
None declared.

References

1 Libby P. Inflammation in atherosclerosis. Nature 2002;420 (6917):868–874
2 Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med 1999;340(02):115–126
3 Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell 2011;145(03):341–355
4 Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. Circ Res 2016;118(04):692–702
5 Vorchheimer DA, Becker R. Platelets in atherothrombosis. Mayo Clin Proc 2006;81(01):59–68
6 Samara WM, Gurbel PA. The role of platelet receptors and adhesion molecules in coronary artery disease. Coron Artery Dis 2003;14(01):65–79
7 van Gils JM, Zwaginga JJ, Hordijk PL. Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. J Leukoc Biol 2009;85(02):195–204
8 Jennings LK. Mechanisms of platelet activation: need for new strategies to protect against platelet-mediated atherothrombosis. Thromb Haemost 2009;102(02):248–257
9 Roberts HR, Monroe DM, Escobar MA. Current concepts of hemostasis: implications for therapy. Anesthesiology 2004; 100(03):722–730
10 Willoughby S, Holmes A, Loscalzo J. Platelets and cardiovascular disease. Eur J Cardiovasc Nurs 2002;1(04):273–288
11 Rosing J, van Rijn JL, Bevers EM, van Dieijen G, Comfurius P, Zwaal RF. The role of activated human platelets in prothrombin and factor X activation. Blood 1985;65(02):319–332
12 Roffi M, Patrono C, Collet JP, et al; ESC Scientific Document Group. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J 2016;37(03):267–315
13 Ibanez B, James S, Agewall S, et al; ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the Management of Acute Myocardial Infarction in Patients Presenting with ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J 2018;39(02):119–177
14 Steg PG, Huber K, Andreotti F, et al. Bleeding in acute coronary syndromes and percutaneous coronary interventions: position paper by the Working Group on Thrombosis of the European Society of Cardiology. Eur Heart J 2011;32(15):1854–1864
15 Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. Circulation 2010;122(18):1837–1845
16 Ionita MG, van den Borne P, Catanzariti LM, et al. High neutrophil numbers in human carotid atherosclerotic plaques are associated with characteristics of rupture-prone lesions. Arterioscler Thromb Vasc Biol 2010;30(09):1842–1848
17 Mangold A, Alias S, Scherz T, et al. Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. Circ Res 2015;116(07):1182–1192

18 Riegger J, Byrne RA, Joner M, et al; Prevention of Late Stent Thrombosis by an Interdisciplinary Global European Effort (PRESTIGE) Investigators. Histopathological evaluation of thrombus in patients presenting with stent thrombosis. A multicenter European study: a report of the prevention of late stent thrombosis by an interdisciplinary global European effort consortium. Eur Heart J 2016;37(19):1538–1549

19 Silvestre-Roig C, Braster Q, Wichapong K, et al. Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death. Nature 2019;569(7755):236–240

20 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

21 Merhi Y, Guidoin R, Provost P, Leung TK, Lam JY. Increase of neutrophil adhesion and vasoconstriction with platelet deposition after deep arterial injury by angioplasty. Am Heart J 1995; 129(03):445–451

22 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

23 Knight JS, Luo W, O'Dell AA, et al. Peptidylarginine deiminase inhibition reduces vascular damage and modulates innate immune responses in murine models of atherosclerosis. Circ Res 2014;114(06):947–956

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

25 Pircher J, Czermak T, Ehrlich A, et al. Cathelicidins prime platelets to mediate arterial thrombosis and tissue inflammation. Nat Commun 2018;9(01):1523

26 Novotny J, Chandraratne S, Weinberger T, et al. Histological comparison of arterial thrombi in mice and men and the influence of Cl-amidine on thrombus formation. PLoS One 2018;13(01):e0190728

27 Ridker PM, Everett BM, Thuren T, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017;377(12):1119–1131

28 Massberg S, Brand K, Grüner S, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J Exp Med 2002;196(07):887–896

29 Sreeramkumar V, Adrover JM, Ballesteros I, et al. Neutrophils scan for activated platelets to initiate inflammation. Science 2014;346(6214):1234–1238

30 Furman MI, Benoit SE, Barnard MR, et al. Increased platelet reactivity and circulating monocyte-platelet aggregates in patients with stable coronary artery disease. J Am Coll Cardiol 1998;31(02):352–358

31 Kopp CW, Gremmel T, Steiner S, et al. Platelet-monocyte cross talk and tissue factor expression in stable angina vs. unstable angina/non ST-elevation myocardial infarction. Platelets 2011; 22(07):530–536

32 Ott I, Neumann FJ, Gawaz M, Schmitt M, Schömig A. Increased neutrophil-platelet adhesion in patients with unstable angina. Circulation 1996;94(06):1239–1246

33 Rossaint J, Zarbock A. Platelets in leucocyte recruitment and function. Cardiovasc Res 2015;107(03):386–395

34 Neumann FJ, Marx N, Gawaz M, et al. Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. Circulation 1997;95(10):2387–2394

35 Brandt E, Petersen F, Ludwig A, Ehlert JE, Bock L, Flad HD. The beta-thromboglobulins and platelet factor 4: blood platelet-derived CXC chemokines with divergent roles in early neutrophil regulation. J Leukoc Biol 2000;67(04):471–478

36 von Hundelshausen P, Petersen F, Brandt E. Platelet-derived chemokines in vascular biology. Thromb Haemost 2007;97(05):704–713

37 Mause SF, von Hundelshausen P, Zernecke A, Koenen RR, Weber C. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. Arterioscler Thromb Vasc Biol 2005;25(07):1512–1518

38 Yuan Y, Alwis I, Wu MCL, et al. Neutrophil macroaggregates promote widespread pulmonary thrombosis after gut ischemia. Sci Transl Med 2017;9(409):eaam5861

39 Looney MR, Nguyen JX, Hu Y, Van Ziffle JA, Lowell CA, Matthay MA. Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion-related acute lung injury. J Clin Invest 2009;119(11):3450–3461

40 Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. J Clin Invest 2006;116(12):3211–3219

41 Ortiz-Muñoz G, Mallavia B, Bins A, Headley M, Krummel MF, Looney MR. Aspirin-triggered 15-epi-lipoxin A4 regulates neutrophil-platelet aggregation and attenuates acute lung injury in mice. Blood 2014;124(17):2625–2634

42 Hamid U, Krasnodembskaya A, Fitzgerald M, et al. Aspirin reduces lipopolysaccharide-induced pulmonary inflammation in human models of ARDS. Thorax 2017;72(11):971–980

43 Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 2013;13(03): 159–175

44 Yang J, Furie BC, Furie B. The biology of P-selectin glycoprotein ligand-1: its role as a selectin counterreceptor in leukocyte-endothelial and leukocyte-platelet interaction. Thromb Haemost 1999;81(01):1–7

45 Evangelista V, Manarini S, Sideri R, et al. Platelet/polymorphonuclear leukocyte interaction: P-selectin triggers protein-tyrosine phosphorylation-dependent CD11b/CD18 adhesion: role of PSGL-1 as a signaling molecule. Blood 1999;93(03): 876–885

46 Evangelista V, Pamuklar Z, Piccoli A, et al. Src family kinases mediate neutrophil adhesion to adherent platelets. Blood 2007; 109(06):2461–2469

47 Théorêt JF, Yacoub D, Hachem A, Gillis MA, Merhi Y. P-selectin ligation induces platelet activation and enhances microaggregate and thrombus formation. Thromb Res 2011;128(03): 243–250

48 Falati S, Gross P, Merrill-Skoloff G, Furie BC, Furie B. Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. Nat Med 2002;8(10): 1175–1181

49 Denis CV, Wagner DD. Platelet adhesion receptors and their ligands in mouse models of thrombosis. Arterioscler Thromb Vasc Biol 2007;27(04):728–739

50 Schmitt C, Abt M, Ciorciaro C, et al. First-in-man study with inclacumab, a human monoclonal antibody against P-selectin. J Cardiovasc Pharmacol 2015;65(06):611–619

51 Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. N Engl J Med 2017;376 (05):429–439

52 Stähli BE, Gebhard C, Duchatelle V, et al. Effects of the P-selectin antagonist inclacumab on myocardial damage after percutaneous coronary intervention according to timing of infusion: insights from the SELECT-ACS trial. J Am Heart Assoc 2016;5(11): e004255

53 Tardif JC, Tanguay JF, Wright SR, et al. Effects of the P-selectin antagonist inclacumab on myocardial damage after percutaneous coronary intervention for non-ST-segment elevation myocardial infarction: results of the SELECT-ACS trial. J Am Coll Cardiol 2013;61(20):2048–2055

54 Oostingh GJ, Pozgajova M, Ludwig RJ, et al. Diminished thrombus formation and alleviation of myocardial infarction and reperfusion injury through antibody- or small-molecule-mediated inhibition of selectin-dependent platelet functions. Haematologica 2007;92(04):502–512

55 Li J, Kim K, Jeong SY, et al. Platelet protein disulfide isomerase promotes glycoprotein Ibα-mediated platelet-neutrophil interactions under thromboinflammatory conditions. Circulation 2019;139(10):1300–1319

56 Hirahashi J, Hishikawa K, Kaname S, et al. Mac-1 (CD11b/CD18) links inflammation and thrombosis after glomerular injury. Circulation 2009;120(13):1255–1265

57 Wang Y, Gao H, Shi C, et al. Leukocyte integrin Mac-1 regulates thrombosis via interaction with platelet GPIbα. Nat Commun 2017;8:15559

58 Bennett JS. Structure and function of the platelet integrin alphaIIbbeta3. J Clin Invest 2005;115(12):3363–3369

59 Estevez B, Du X. New concepts and mechanisms of platelet activation signaling. Physiology (Bethesda) 2017;32(02): 162–177

60 Pluskota E, Woody NM, Szpak D, et al. Expression, activation, and function of integrin alphaMbeta2 (Mac-1) on neutrophil-derived microparticles. Blood 2008;112(06):2327–2335

61 Jamasbi J, Ayabe K, Goto S, Nieswandt B, Peter K, Siess W. Platelet receptors as therapeutic targets: past, present and future. Thromb Haemost 2017;117(07):1249–1257

62 Firbas C, Siller-Matula JM, Jilma B. Targeting von Willebrand factor and platelet glycoprotein Ib receptor. Expert Rev Cardiovasc Ther 2010;8(12):1689–1701

63 Gresele P, Momi S. Inhibitors of the interaction between von Willebrand factor and platelet GPIb/IX/V. Handb Exp Pharmacol 2012;(210):287–309

64 Lei X, Reheman A, Hou Y, et al. Anfibatide, a novel GPIb complex antagonist, inhibits platelet adhesion and thrombus formation in vitro and in vivo in murine models of thrombosis. Thromb Haemost 2014;111(02):279–289

65 Schulz C, von Brühl ML, Barocke V, et al. EMMPRIN (CD147/basigin) mediates platelet-monocyte interactions in vivo and augments monocyte recruitment to the vascular wall. J Thromb Haemost 2011;9(05):1007–1019

66 Kuijper PH, Gallardo Tores HI, Lammers JW, Sixma JJ, Koenderman L, Zwaginga JJ. Platelet associated fibrinogen and ICAM-2 induce firm adhesion of neutrophils under flow conditions. Thromb Haemost 1998;80(03):443–448

67 Faraday N, Schunke K, Saleem S, et al. Cathepsin G-dependent modulation of platelet thrombus formation in vivo by blood neutrophils. PLoS One 2013;8(08):e71447

68 Horn M, Bertling A, Brodde MF, et al. Human neutrophil alpha-defensins induce formation of fibrinogen and thrombospondin-1 amyloid-like structures and activate platelets via glycoprotein IIb/IIIa. J Thromb Haemost 2012;10(04):647–661

69 Kaiser P, Harenberg J, Walenga JM, et al. Effects of a heparin-binding protein on blood coagulation and platelet function. Semin Thromb Hemost 2001;27(05):495–502

70 Wohner N, Keresztes Z, Sótonyi P, et al. Neutrophil granulocyte-dependent proteolysis enhances platelet adhesion to the arterial wall under high-shear flow. J Thromb Haemost 2010;8(07): 1624–1631

71 Gorudko IV, Sokolov AV, Shamova EV, et al. Myeloperoxidase modulates human platelet aggregation via actin cytoskeleton reorganization and store-operated calcium entry. Biol Open 2013;2(09):916–923

72 Kolarova H, Klinke A, Kremserova S, et al. Myeloperoxidase induces the priming of platelets. Free Radic Biol Med 2013; 61:357–369

73 LaRosa CA, Rohrer MJ, Benoit SE, Rodino LJ, Barnard MR, Michelson AD. Human neutrophil cathepsin G is a potent platelet activator. J Vasc Surg 1994;19(02):306–318, discussion 318–319

74 Gould TJ, Vu TT, Swystun LL, et al. Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms. Arterioscler Thromb Vasc Biol 2014;34(09):1977–1984

75 Jordan RE, Nelson RM, Kilpatrick J, Newgren JO, Esmon PC, Fournel MA. Inactivation of human antithrombin by neutrophil elastase. Kinetics of the heparin-dependent reaction. J Biol Chem 1989;264(18):10493–10500

76 Wang Y, Fang C, Gao H, et al. Platelet-derived S100 family member myeloid-related protein-14 regulates thrombosis. J Clin Invest 2014;124(05):2160–2171

77 Pruenster M, Kurz AR, Chung KJ, et al. Extracellular MRP8/14 is a regulator of β2 integrin-dependent neutrophil slow rolling and adhesion. Nat Commun 2015;6:6915

78 Altwegg LA, Neidhart M, Hersberger M, et al. Myeloid-related protein 8/14 complex is released by monocytes and granulocytes at the site of coronary occlusion: a novel, early, and sensitive marker of acute coronary syndromes. Eur Heart J 2007;28(08): 941–948

79 Rossaint J, Kühne K, Skupski J, et al. Directed transport of neutrophil-derived extracellular vesicles enables platelet-mediated innate immune response. Nat Commun 2016;7:13464

80 Paul BZ, Jin J, Kunapuli SP. Molecular mechanism of thromboxane A(2)-induced platelet aggregation. Essential role for p2t(ac) and alpha(2a) receptors. J Biol Chem 1999;274(41):29108–29114

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

82 Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol 2007;176(02): 231–241

83 Wang Y, Li M, Stadler S, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol 2009;184(02):205–213

84 Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med 2010;207(09):1853–1862

85 Leshner M, Wang S, Lewis C, et al. PAD4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures. Front Immunol 2012;3:307

86 Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol 2010;191(03):677–691

87 Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 2018;18(02):134–147

88 Pilsczek FH, Salina D, Poon KK, et al. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. J Immunol 2010;185(12):7413–7425

89 Martinod K, Wagner DD. Thrombosis: tangled up in NETs. Blood 2014;123(18):2768–2776

90 Megens RT, Vijayan S, Lievens D, et al. Presence of luminal neutrophil extracellular traps in atherosclerosis. Thromb Haemost 2012;107(03):597–598

91 Borissoff JI, Joosen IA, Versteylen MO, et al. Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. Arterioscler Thromb Vasc Biol 2013;33(08):2032–2040

92 de Boer OJ, Li X, Teeling P, et al. Neutrophils, neutrophil extracellular traps and interleukin-17 associate with the organisation of thrombi in acute myocardial infarction. Thromb Haemost 2013;109(02):290–297

93 Stakos DA, Kambas K, Konstantinidis T, et al. Expression of functional tissue factor by neutrophil extracellular traps in culprit artery of acute myocardial infarction. Eur Heart J 2015; 36(22):1405–1414

94 Martinod K, Demers M, Fuchs TA, et al. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. Proc Natl Acad Sci U S A 2013; 110(21):8674–8679

95 Franck G, Mawson TL, Folco EJ, et al. Roles of PAD4 and NETosis in experimental atherosclerosis and arterial injury: implications for superficial erosion. Circ Res 2018;123(01):33–42

96 Noubouossie DF, Whelihan MF, Yu YB, et al. In vitro activation of coagulation by human neutrophil DNA and histone proteins but not neutrophil extracellular traps. Blood 2017;129(08):1021–1029

97 Kannemeier C, Shibamiya A, Nakazawa F, et al. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. Proc Natl Acad Sci U S A 2007;104(15):6388–6393
98 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
99 Semeraro F, Ammollo CT, Morrissey JH, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. Blood 2011;118(07):1952–1961
100 Ammollo CT, Semeraro F, Xu J, Esmon NL, Esmon CT. Extracellular histones increase plasma thrombin generation by impairing thrombomodulin-dependent protein C activation. J Thromb Haemost 2011;9(09):1795–1803
101 Michels A, Albánez S, Mewburn J, et al. Histones link inflammation and thrombosis through the induction of Weibel-Palade body exocytosis. J Thromb Haemost 2016;14(11):2274–2286
102 Laridan E, Denorme F, Desender L, et al. Neutrophil extracellular traps in ischemic stroke thrombi. Ann Neurol 2017;82(02): 223–232
103 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
104 Østerud B, Bjørklid E. Sources of tissue factor. Semin Thromb Hemost 2006;32(01):11–23
105 Day SM, Reeve JL, Pedersen B, et al. Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. Blood 2005;105(01):192–198
106 Kambas K, Mitroulis I, Apostolidou E, et al. Autophagy mediates the delivery of thrombogenic tissue factor to neutrophil extracellular traps in human sepsis. PLoS One 2012;7(09):e45427
107 Pfeiler S, Stark K, Massberg S, Engelmann B. Propagation of thrombosis by neutrophils and extracellular nucleosome networks. Haematologica 2017;102(02):206–213
108 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
109 Tadie JM, Bae HB, Jiang S, et al. HMGB1 promotes neutrophil extracellular trap formation through interactions with Toll-like receptor 4. Am J Physiol Lung Cell Mol Physiol 2013;304(05): L342–L349
110 Vogel S, Bodenstein R, Chen Q, et al. Platelet-derived HMGB1 is a critical mediator of thrombosis. J Clin Invest 2015;125(12): 4638–4654
111 Bianchi ME, Beltrame M. Upwardly mobile proteins. Workshop: the role of HMG proteins in chromatin structure, gene expression and neoplasia. EMBO Rep 2000;1(02):109–114
112 Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol 2010; 28:367–388
113 Rath D, Geisler T, Gawaz M, Vogel S. HMGB1 expression level in circulating platelets is not significantly associated with outcomes in symptomatic coronary artery disease. Cell Physiol Biochem 2017;43(04):1627–1633
114 Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat Genet 2012;44(11):1179–1181
115 Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017; 377(02):111–121
116 Wolach O, Sellar RS, Martinod K, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci Transl Med 2018;10(436):eaan8292
117 Hobbs CM, Manning H, Bennett C, et al. JAK2V617F leads to intrinsic changes in platelet formation and reactivity in a knock-in mouse model of essential thrombocythemia. Blood 2013;122 (23):3787–3797