Effect of neutrophil extracellular traps (NET) on thrombosis: A literature review

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ABSTRACT

Neutrophils are used by the innate immune system to form thrombi during many types of thrombosis. The inactivation of endogenous anticoagulants results in increased intravascular coagulation by increasing factor XII activation, decreasing plasmin production, or boosting the tissue factor-dependent extrinsic pathway. The creation of neutrophil extracellular traps (NET) by the externalization of decondensed nucleosomes and granule proteins supports neutrophil-dependent prothrombotic processes. Experimental thrombosis has been demonstrated to be caused by these traps, whether intact or fragmented, since they provide the means to enhance microvascular thrombosis. The von Willebrand factor increases platelet adhesion when neutrophils and activated platelets combine to form NET. Thus, neutrophils and externalized nucleosomes promote intravascular blood coagulation and thrombosis during infections and during conditions resulting from blood vessel damage.

Key Words: neutrophil, neutrophil extracellular traps, NET, thrombosis.

INTRODUCTION

An infection with a pathogen or sterile damage to tissue triggers the recruitment of neutrophils as the first immune cell. Microbicidal activity of neutrophils is involved in preventing the spread of pathogens throughout the body and the prevention of pathogen infection.\(^1,^2\) A neutrophil extracellular trap (NET) is secreted by neutrophils as a host defence against pathogens. NETs primarily consist of decondensed nucleosomes and intracellular proteins, including neutrophil elastase (NE) and myeloperoxidase.\(^1,^3\)

Under specific situations, the activation of intravascular blood clotting and the development of thrombi promote a separate intravascular immunity mechanism known as immunothrombosis.\(^2\) This biological thrombosis immobilizes circulating bacteria, restricts tissue invasion, and reduces circulating bacteria survival in organs such as the liver and spleen. In mice, neutrophil extracellular traps were identified as the primary effectors of intravascular immunity supported by microvascular thrombosis.\(^4,^5\)

This study outlines the mechanisms that promote thrombosis propagation by neutrophils, how NETs or nucleosomes are externalized at the cellular level, and underlines the critical role of neutrophils in the activation of diverse forms of thrombosis in vivo.

THROMBOSIS

In physiological conditions, the hemostasis process reflects the balance of interactions between factors that trigger and inhibit blood clotting. However, under certain conditions, the physiological process of normal hemostasis can at any time cause pathological thrombosis, causing arterial or venous occlusion. It is not uncommon for various therapeutic interventions that are commonly used in patient management to change the thrombotic hemostatic balance so that it becomes pathological.\(^2,^6\)

The process of thrombosis involves interactions between three main factors, namely blood vessel walls, coagulation proteins, and platelets.\(^1,^3\) A disturbance in the balance between factors that stimulate and prevent thrombosis can cause thrombus formation. There are many acute vascular diseases that are commonly caused by thrombus formation in blood vessels, such as myocardial infarction, thrombotic cerebrovascular disease, and venous thrombosis. Although in their pathophysiology these diseases both have the final result in the form of blood vessel occlusion and tissue ischemia, the processes that regulate these pathological conditions have different mechanisms.\(^1,^6\)

Arterial thrombi are formed through a series of sequential steps consisting of the processes of platelet adhesion to the blood vessel wall, platelet aggregation, and thrombin activation. Damage to the vascular endothelium stimulates platelet activation which then causes adhesion to
von Willebrand factor and collagen. This adhesion causes platelet activation, shape changes, as well as the synthesis and release of thromboxane A2, serotonin 5-HT receptors, and ADP. Platelet stimulation also causes conformational changes in the glycoprotein IIb-IIIa integrin receptor, causing high-affinity fibrinogen binding and stable thrombus formation. In venous thrombosis, the mechanism of occurrence can be caused by primary hypercoagulability which describes a defect in the proteins regulating coagulation and/or fibrinolysis, or secondary hypercoagulability can also occur due to abnormalities in blood vessels and blood flow, causing thrombosis.6,7

**NEUTROPHIL EXTRACELLULAR TRAPS (NET)**

Neutrophil extracellular traps (NET) are extracellular traps composed of most of the chromatin of neutrophil granular proteins such as neutrophil elastase (NE), myeloperoxidase (MPO), and cathepsin G.14-16 NETs will trap bacteria and simultaneously provide support for local anti-microbial components so that they can kill extracellular microbes. The formation of NETs, called NETosis, can destroy the neutrophils themselves and subsequently lead to chromatin decondensation, nuclear enlargement, and membrane perforation.6,8 First, the nuclei will lose their characteristic lobular shape and enlarge. This nuclear enlargement process is caused by chromatin decondensation mediated by the enzyme peptidylarginine deiminase-4 (PAD4).9 PAD4 is an enzyme that citrullinates proteins by entering the nucleus to modify histones.10,11 During the hyper citrullination process, specific arginine residues on histone H3 and H4, there is a loss of positive charge due to the transformation of arginine residues, histone H1 and heterochromatin protein 1β apart from the nucleosome structure. in vitro is said to take approximately <30 to 240 minutes depending on the type and concentration of stimulus, the neutrophil isolation procedure, and the sensitivity of the detection method.12 Neutrophil elastase (NE) also requires PAD4 to digest nucleosomal histones.13,14 Simultaneously with this, the nuclear membrane begins to decondensate and the neutrophil granules begin to be destroyed. Thus, granule proteins, such as MPO, will come into contact with nuclear components such as chromatin. MPO is the biggest trigger of NETosis apart from PAD4 mediation.15

The chemicals implicated in NETosis are still unknown. The inclusion of other intracellular membranes is enabled by the disintegration of nuclei and granules. These cells combine, resulting in chromatin decondensation, which is released together with numerous other granule components to produce NETs. This NET trap is usually found in gram-positive bacteria and gram-negative bacteria which play a role in vitro microbial activity, fungal infections and viral infections.13,14 NETs are intact chromatin fiber scaffolds with antimicrobial proteins, ideal for retaining a large number of microbes. Therefore, some bacterial pathogens have evolved to express deoxyribonuclease (DNase), which is capable of eliminating NETs and conferring virulence.13,15 While references to NETs in fighting pathogens have been widely reported pathological formation of NETs is starting to appear in reports.13,16,17 NETosis has been observed in several diseases without a triggering microorganism infection such as pre-eclampsia, small vessel vasculitis, systemic lupus erythematosus (SLE) and associated nephritis.17-19 Destruction of serum deoxyribonucleases (DNases) drives the pathogenesis of SLE, leading to the production of antibodies against its own DNA.20 There is a benefit from intravascular NETosis in sepsis conditions where this NETosis is protective for the host against bacteremia.18,19 Large amounts of antimicrobial toxic products will release NETs, several special histones, the main protein components of NETs, which can contribute to death in sepsis.14,18 This suggests that there is only a small threshold between the beneficial and harmful effects of NET on infected hosts.11,14,19 NETs not only attract pathogens, but can also bind to platelets and red blood cells. Because red blood cells rich in thrombi are formed in deep veins, NETs were first discovered in deep vein thrombosis (DVT).20 Infection is one of the factors the risk of DVT is possible through NET formation. The relationship between NETosis and coagulation is formed due to the presence of NE in NETosis. Increased procoagulant activity is the result of NE's cleavage of the tissue factor pathway inhibitor (TFPI). Platelet activation is triggered by procoagulant activity, and it can lead to an increase in NET formation. However, NETosis activity cannot be prevented by platelet depletion.19-22

**NEUTROPHIL EXTRACELLULAR TRAPS (NET) IN THROMBOSIS**

NETs have been investigated in arterial vascular injuries precipitated by ferric chloride application. In this study, lack of serine proteases in NE or deficiency of cathepsin-G reduced coagulation through reduced cleavage of TFPI.21 Addition of anti-H2A-H2B-DNA antibodies capable of neutralizing histones resulted in prolonged time to occlusion and lower thrombus stability in type 2 carotid arteries. wild (WT) mice, where no effect was found from the addition of antibodies to NE or cathepsin-G deficiency in mice.21 External nucleosomes thus contribute to thrombogenesis by exposing serine proteases to TFPI. NETs were also found in the carotid lumen of ApoE-deficient mice fed a high-fat diet, ranging from proximal to atherosclerotic lesions, lending credence to the clinical observation that NETs play a role in coronary atherosclerosis.23 Platelets and neutrophils are the main components observed in the model of inferior vena cava stenosis in rats experiencing deep vein thrombosis (DVT), as well as Von Willebrand Factor (VWF) which may be closely related to these two components.23,24 Release of VWF from Weibel- Palade bodies in endothelial cells are initiated by hypoxia. Ischemic stroke increases plasma levels of nucleosomes and causes systemic hypoxia by releasing histone-DNA complexes into the circulation. After experiencing hypoxia, mice were observed to be highly susceptible to intravascular thrombus formation. Hypoxia induces hypoxia-inducible factor 1α (HIF-1α), which is involved in NETosis.23,24 NETs will then increase endothelial activation via histones, where the addition of histones in intravascular stenosis further accelerates thrombus formation. The addition of histones will trigger the release of VWF and give rise to signs of micro thrombosis.
in mice. Secretion of Weibel-Palade bodies also upregulates endothelial P-selectin, an adhesion receptor for leukocytes.12,23-25

Thrombus leukocytes are present in neutrophils, which are among the first leukocyte components to be recruited during the activation of the endothelium at the onset of thrombosis.25,26 Many references state that the presence of NETs in DVT is related, but the role of NETs in the pathophysiology of DVT itself is still being studied further. In treatment with DNase 1 is known to reduce NETs, thereby reducing the frequency of thrombus formation. This indicates that the presence of NETs in DVT is quite important functionally and DNase treatment is therapeutically useful. At some time after the occurrence of intravascular stenos, several NETs appear along with thrombus formation as can be seen on intravital microscopy, where at some time later, the NETs appear to spread.26 Risk factors for DVT such as trauma, surgery, infection, immobilization and hypoxia, are related to with NET formation.25,26 DVT and pulmonary embolism have specific locations where accumulation occurs.

NEUTROPHIL EXTRACELLULAR TRAPS (NET) AS A MARKER AND STRATEGY FOR THERAPEUTIC TARGETING OF THROMBOSIS

There are several potential new targets to prevent thrombosis or improve thrombolysis. Platelets and platelet adhesion to VWF are essential processes in thrombus formation, and randomized clinical trial research suggests that aspirin, which is known as antiplatelet therapy, is able to prevent recurrence of thromboembolic events.27 Interestingly, aspirin can inhibit NETosis in vitro. Prevention of the release of Weibel-Palade bodies with therapeutic agents capable of increasing nitric oxide (NO) formation, disrupting endothelial VWF/P-selectin secretion, or targeting platelet-VWF interactions is said to prevent platelets and neutrophils from anchoring to blood vessel walls. and the possible recruitment of these two components to other platelet-neutrophil activation. The A1 domain in VWF binds 1β glycoprotein on platelets, which will support the platelet-neutrophil adhesion interaction.23,27,28

Significantly less thrombus forms in veins and arteries when the VWF A1 domain interaction is inhibited in vivo by aptamers or antibodies. Leukocyte rolling caused by P-selectin glycoprotein ligand-1 (PSGL-1) and adhesion via a2 integrin, such as Mac1, will be inhibited by blocking VWF.22,25,27 ADAMTS13, a protease that cleaves VWF specifically, may be involved in reducing thrombosis and improving thrombolysis of thrombi in venules in vivo.

As ischemia reperfusion takes place, recombinant ADAMTS13 (rADAMTS13) may stop microthrombosis from happening, in stroke and myocardial infarction. By lowering initial platelet accumulation and neutrophil recruitment, as well as by enhancing thrombolysis when combined with fibrinolytics or therapy for NET removal, rADAMTS13 can also be used as a preventative measure for DVT. Combining ADAMTS13 and DNase I has been shown to improve human myocardial infarction activity and to reduce some of its associated symptoms.27,28 Serine protease increases DNase 1 activity on chromatin in vitro; heparin can imitate this action by removing histones from chromatin and facilitating easier enzyme access. Heparin and DNase 1 together may lower the chance of thrombotic episodes in the future.25,27

As it can lower neutrophil recruitment, active platelet-neutrophil interactions, and the formation of TF-containing microparticles, targeting P-selectin and other crucial components of Weibel-Palade bodies and -granules is a very beneficial approach. Reduced neutrophil recruitment to the blood vessel wall, NET formation, and P-selectin’s procoagulant activity are all markedly decreased when P-selectin is inhibited in animal models of DVT.23,25,28

CONCLUSION

The main process by which neutrophils generate fibrin and activate platelets is by extruding extracellular nucleosomes, or NETs. Since platelets and neutrophils both encourage the formation of NETs and extracellular chromatin activates their interaction, platelets play a role in thrombosis, which is controlled by neutrophils. All things considered, this shows that NETs and neutrophils may play a role in thrombotic cardiovascular diseases like myocardial infarction, stroke, and venous thromboembolism.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

All of the authors equally contributed to the study.

REFERENCES


