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● ● ● THROMBOSIS AND HEMOSTASIS

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Demystifying the prothrombotic role of NETs

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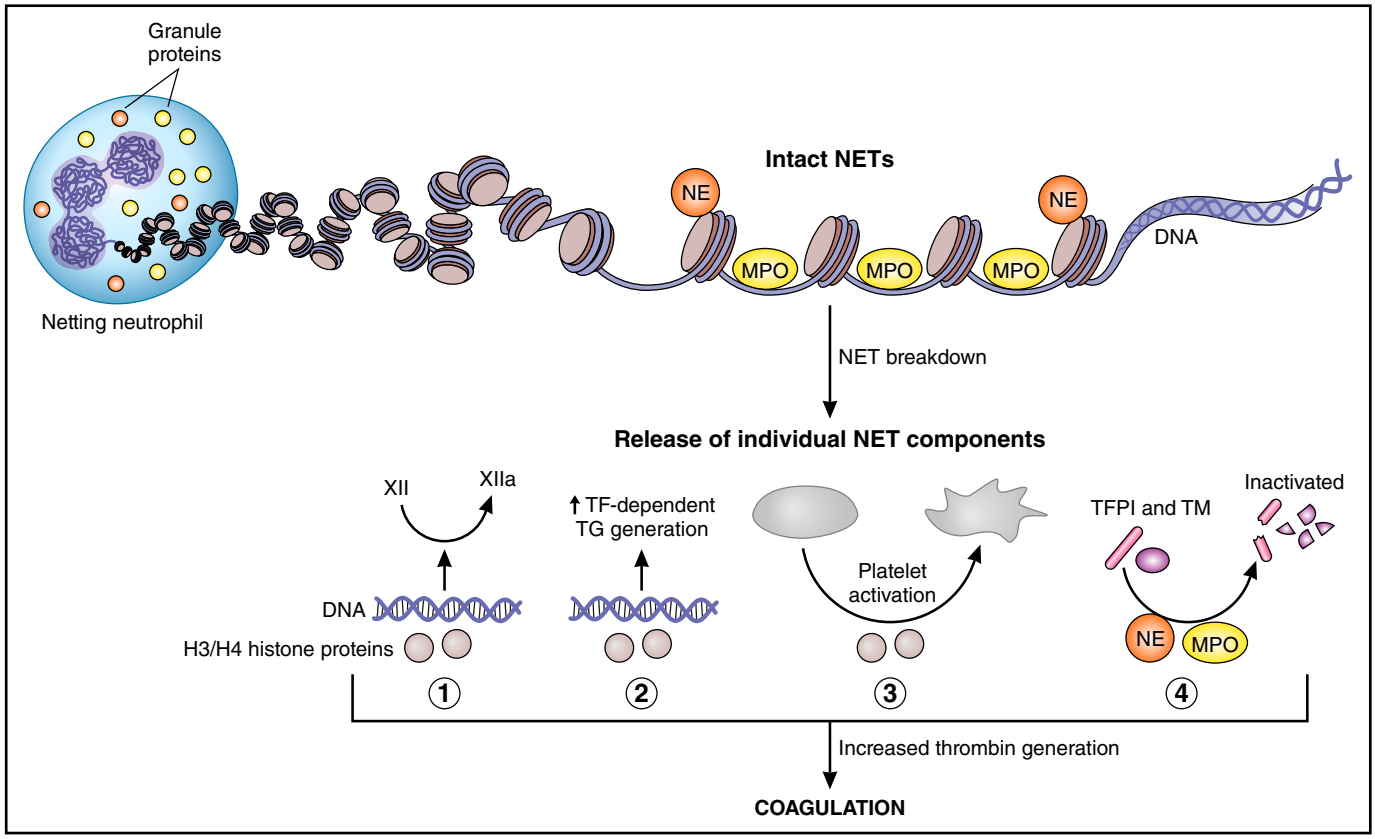
In this issue of *Blood*, Noubouossie and colleagues report surprising findings on the role of neutrophil-derived nuclear material in blood coagulation. The authors provide evidence that, in contrast to DNA and histone proteins, neutrophil extracellular traps (NETs) do not contribute directly to coagulation of human plasma. These findings implicate differential functions of nuclear material in thrombosis and are of importance for the development of antithrombotic therapies targeting NETs.[1]

Innate immune cells play an important role in blood coagulation by releasing prothrombotic molecular cues. Monocytes and their microvesicles provide tissue factor, which initiates the extrinsic pathway of coagulation. Neutrophils release, among other factors, peroxidase and proteases, which inactivate anticoagulants such as tissue factor pathway inhibitor and thrombomodulin to promote blood coagulation. These prothrombotic pathways culminate in the generation of fibrin, the end product of the coagulation cascade. Fibrin provides an intravascular scaffold to trap and eliminate pathogens.[2] Propagation of blood coagulation by innate immune cells therefore contributes to intravascular immunity and is now recognized as an integral part of host defense.[3]

In addition to soluble molecules, activated neutrophils release nuclear material in form of chromatin lattices, known as NETs.[4] Extracellular nucleosomes consist of DNA wound around histone proteins reaching variable diameters dependent on the extent of chromatin decondensation. In addition, NETs are decorated with antimicrobial peptides and proteases derived from neutrophil cytoplasmic granules. They contribute to the elimination of bacteria and fungi, which bind directly to NETs and are exposed to a variety of antimicrobial defenses.[4] NETs thereby contribute to establish an intravascular scaffold for the containment and elimination of pathogens. Importantly, NET formation not only contributes to host defense but also has been associated with cardiovascular diseases and other clinical conditions. In fact, NETs are present in arterial thrombi of patients with myocardial infarction and in thrombus specimens retrieved from the venous circulation.[5] Inhibition of NET formation or dismantling NETs by DNase treatment reduces thrombosis in mice.[6,7] Thus, targeting NETs has evolved as an interesting strategy for the treatment of thrombotic conditions. However, the precise contribution to clot formation of the nuclear elements comprising NETs has been unclear.

Noubouossie et al addressed the differential effects of purified NET components on the coagulation of human plasma in vitro. They report that purified DNA not only activates the intrinsic pathway of coagulation through FXII but also amplifies tissue factor–dependent thrombin generation. Further, single histones induce thrombin generation in a platelet-dependent manner (see figure). In contrast, intact NETs have no procoagulant effect in vitro, and purified DNA loses its procoagulant activity when histones are added.[1] This is surprising, since studies in mice provided evidence that NET destruction represents an efficient antithrombotic strategy.[7,8] Why should intact NETs be less thrombogenic? The authors speculate that this could be due to neutralization of the negative charge of DNA on the NET surface. Pending further proof under these experimental conditions, this concept is not unlikely, since histones provide a positive net charge, and charge–charge interactions may result in its neutralization.[9] This could limit activation of the contact system and thrombin generation. Interestingly, the antimicrobial activity of NETs depends on its negative charge. Neutralizing this property by providing excess cations reduces bacterial killing.[10]

The report of Noubouossie et al therefore provides an important contribution to our understanding of the role of neutrophil nuclear material in blood coagulation. It suggests that therapeutic strategies targeting NETs should be directed against specific NET structures such as histone proteins, DNA, or NET-bound serine proteases, which give free rein to blood coagulation by proteolysis of the coagulation suppressor tissue factor pathway inhibitor. Nevertheless, future studies will have to assess whether these findings hold in more complex settings



Activated neutrophils release NETs, which are scaffolds of decondensated DNA and histones. Noubouossie et al report that individual NET components such as free DNA and histone proteins promote thrombin generation (TG) through the following 3 pathways: (1) activation of the contact pathway via factor XII, (2) amplification of tissue factor (TF)–dependent TG, (3) and activation of platelets via histones H3 and H4. (4) In addition, natural anticoagulants such as tissue factor pathway inhibitor (TFPI) and thrombomodulin (TM) can be inactivated by myeloperoxidase (MPO) and serine proteases (ie, neutrophil elastase [NE]). Professional illustration by Patrick Lane, ScEYEnce Studios.

in vitro (ie, whole blood, flow conditions) and especially in vivo.

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