demarcation membrane system starts at focal points of the cell surface. They could capture the very first moments of demarcation membrane formation, which they named the predemarcation membrane system, and determined their 3-dimensional (3D) architecture using dual axis electron tomography and large volume focused ion beam-scanning electron microscopy. Further, the authors found that a growing demarcation membrane system requires, besides invagination of the plasma membrane, insertion of Golgi-derived membrane vesicles and endoplasmic reticulum–demarcation membrane system tethering. Earlier reports showed numerous Golgi stacks targeted to the region of furrow formation during anaphase in mitotic cells, thereby contributing to active membrane delivery. This suggested that as the megakaryocyte increases in size and ploidy, it is also prepared to augment the mass of intracellular membranous territories.

Past studies proposed that the demarcation membranes define platelet territories. Do these membranes play yet unidentified additional roles? In the current study, Eckly et al mapped the location of the predemarcation membranes that start by plasma membrane invaginations, in relation to nuclear material. These membrane structures were observed between the nuclear lobes of polyploid megakaryocytes (see figure), with an intriguing correlation between the number of lobes and the plasma membrane connections. During normal mitosis, the Golgi complexes disassemble and reform during telophase. This process, however, was never studied during megakaryocyte endomitosis. Considering the origin and dynamics of demarcation membrane formation and its localization between nuclear lobes, the authors discuss the interesting hypothesis that these membranes are extended from the Golgi in consortium with endomitosis to aid in control of megakaryocyte endomitosis and polyploidy. What might argue against this contention is the uncoupling reported earlier between the development of demarcation membranes and ploidy acquisition, such as in the case of targeted expression of cyclin D3 to megakaryocytes in vivo, resulting in ploidy level similar to thrombopoietin administration, despite poorer development of demarcation membranes. Further, mutant Gunn mice exhibit abnormal megakaryocyte demarcation membranes but also an increase in ploidy level. Naturally, each of these cases represents abnormal gene expression that might not mirror the situation in normally developing megakaryocytes. Whether or not the demarcation membranes take part in controlling megakaryocyte endomitosis warrants future examination.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Dinarvand et al, page 935

PolyP and APC fight a RAGEing battle
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In this issue of Blood, Dinarvand and colleagues identify polyphosphate (polyP) as a potent mediator of proinflammatory effects induced by nuclear proteins such as histone H4 and high mobility group box 1 (HMGB1). Coagulation, platelet activation, and inflammation are intricately linked and regulatory mechanisms ensure a balanced response to infection and inflammation. Not only do these observations promote polyP to the ranks of an all-round proinflammatory and procoagulant agent, but also protection by activated protein C (APC) against these proinflammatory effects reveals an intricate battle between polyP and APC that is fought on multiple fronts.

Nuclear proteins such as histones and HMGB1 are increasingly recognized to play an important role in infection and inflammation. Released in the circulation via neutrophil extracellular traps, leaking from necrotic cells, or secreted in response to lipopolysaccharide, these nuclear proteins, or nuclear cytokines, are generally proinflammatory and often cytotoxic to cells as they are recognized by pattern-recognition receptors (PRRs) such as the various Toll-like receptors (TLRs) and the receptor for advanced glycation end products (RAGE). In a striking example, histones cause profound platelet activation mediated at least in part by TLRs, resulting in the release of platelet-derived polyP with prohemostatic, prothrombotic, and proinflammatory effects.

Previously, the pathophysiologically achievable concentrations of nuclear proteins in the circulation and the concentrations required for cytotoxicity in experimental models seemed to overlap narrowly at best. However, polyP changes this picture. As proposed by Dinarvand et al, polyP promotes binding of HMGB1 (and histone H4) to RAGE, facilitates clustering of oligomeric receptor complexes to initiate signaling, and amplifies their proinflammatory signaling via ligation with polyP-activated P2Y1 receptors. Consequently, polyP reduces the concentrations of HMGB1 or histone H4 that are required to elicit proinflammatory signaling, suggesting that the contributions of these nuclear cytokines to proinflammatory effects may be much more intricate than thus far appreciated.
Overview of the multiple opposing effects of polyP and APC on inflammation and coagulation. The proinflammatory (top left) and procoagulant (top right) effects of polyP provide multiple head-to-head confrontations with the cytoprotective effects (bottom left) and anticoagulant (bottom right) activities of APC. Amplification by polyP of proinflammatory effects by histones or HMGB1 that require RAGE and P2Y1, are inhibited by APC's cytoprotective effects that require PAR1 and EPCR. Furthermore, APC directly inactivates histones, whereas histones inhibit the generation of APC by the thrombin-thrombomodulin complex (IIa-TM). PolyP also mediates numerous procoagulant effects that include stimulation of factor V (FV) activation by thrombin (IIa) and factor Xa (FXa), inhibition of TFPI function, stimulation of FXI activation by thrombin, and inhibition of fibrinolysis. Procoagulant effects of polyP are counteracted by anticoagulant effects of APC that involve proteolysis of FVas to inactive FVa (FVai). Consequent to FVa inactivation, APC alleviates FVa-dependent inhibition of TFPI by polyP, eliminates FVas as cofactor for FXI activation by thrombin, and promotes fibrinolysis. Not shown are the activation of the contact system resulting in the generation of bradykinin by long-chain polyP and the inactivation of HMGB1 by the thrombin-thrombomodulin complex. Green arrows indicate stimulation/activation; red arrows indicate inhibition/inactivation.

Where does the polyP come from? Infectious microorganisms provide a source of long-chain polyP (up to 2000 phosphate units), whereas activated platelets provide the major source of endogenous medium-chain polyP (60-100 phosphate units). Interestingly, some effects of polyP are restricted by polymer length such as activation of the contact system and generation of proinflammatory bradykinin that require long-chain polyP. Other procoagulant effects of polyP require shorter polymers such as found in platelet-derived polyP. Although long-chain polyP seems more efficient, amplification of proinflammatory effects of HMGB1 or histone H4 occurs by both medium-chain and long-chain polyP, suggesting that medium-chain polyP such as found in platelets is a legitimate proinflammatory mediator. One pertinent question that arises is how the proinflammatory and procoagulant effects of polyP are kept in check as inactivation of polyP by endo- and exopolyporphatas results in a relatively long half-life in the circulation of ~1.5 to 2 hours. Could APC perhaps play a role?

The proinflammatory and procoagulant effects of polyP provide a stark contrast to the anticoagulant and cytoprotective activities of APC and upon closer examination reveal an astonishing battlefield between APC and polyP (see figure). APC is a dual-function protease with direct cytoprotective effects on cells in addition to its anticoagulant effects that are mediated by the proteolytic inactivation of FVas and FVIIIa. APC's cytoprotective activities that require protease activated receptor 1 (PAR1) and the endothelial protein C receptor (EPCR) inhibit proinflammatory signaling of HMGB1 mediated by TLR2, TLR4, and RAGE. In addition, APC inhibits proinflammatory polyP-amplified responses of HMGB1 or histone H4 that are mediated by RAGE and P2Y1. However, APC's stance to histones, HMGB1, and polyP is more elaborate. APC proteolytically inactivates extracellular histones, whereas histones inhibit the formation of APC by the thrombin-thrombomodulin complex. Binding of HMGB1 to thrombomodulin inhibits its proinflammatory effects, and proteolytic cleavage of HMGB1 by the thrombin-thrombomodulin complex greatly diminishes its ability to induce proinflammatory cell signaling. Additionally, an inhibitor screen for polyP highlighted one endogenous molecule of particular interest, namely platelet factor 4 (PF4). This cationic protein released from platelets not only binds tightly to polyP and inhibits its effects but also enhances the formation of APC, thereby highlighting yet another player in the conflict between polyP and APC.

On another front, polyP and APC battle for control of coagulation (see figure). Proteolytic inactivation of FVas by APC directly counteracts the stimulation of FV activation by polyP. Consequent to stimulation of FV activation, polyP promotes FVa-mediated inhibition of tissue factor pathway inhibitor (TFPI) function, whereas inactivation of FVa by APC presumably restores TFPI function. Another important procoagulant effect of polyP resides in its ability to enhance the thrombin-mediated activation of factor XI (FXI), which is counteracted by APC-mediated inactivation of FVa, a recently identified cofactor of FXI activation by thrombin. Finally, inhibition of fibrinolysis by polyP is antagonized by proteolytic effects of APC. Taken together, the sheer number of direct confrontations between the effects of polyP and APC for control of inflammation as well as coagulation is remarkable and provides at least an initial rationale for additional studies, including investigations into the potential synergistic effects of combined polyP inhibition and APC therapy in prothrombotic inflammatory disease.

Our understanding of the role of polyP in coagulation and inflammation has been rapidly evolving in recent years and will no doubt continue to do so in the coming years. Similarly, insights into the protein C system experienced exponential growth in past years as have studies unraveling the role of nuclear cytokines and their interactions with PRRs. Intertwining these popular and rapidly accelerating fields, as demonstrated in the article by Dinarvand et al, provides an important novel conceptual advance to understanding the intricate mechanisms responsible for a balanced and sometimes unbalanced response to infection and inflammation. Furthermore, many new questions are raised that provide important fuel for exciting basic and translational research hypotheses for a variety of diseases where coagulation and inflammation contribute to pathogenesis.

Conflict-of-interest disclosure: The author declares no competing financial interests.
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