EUROPEAN HEMATOLOGY ASSOCIATION

Thrombosis - Section 1

Cross-talk between inflammation and coagulation

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Take-home messages

- Coagulation and inflammation are closely linked and the FXII-driven contact activation system is an example for this intimate crosstalk.
- Platelet-released polyP forms nanoparticles that are retained on platelet surfaces where they directly initiate FXII activation..
- A routine assay for platelet polyP exists and may be used to establish the polymer as a biomarker for thrombosis.
- Targeting polyP protects from thrombosis in an FXII-dependent manner in vivo without elevating the bleeding risk.

Introduction

Coagulation and inflammation are considered as two distinct pathologies but they closely interact at multiple levels. The end result of proinflammatory and procoagulant reactions constitutes the unifying principle for a variety of disorders affecting the cardiovascular system including atherothrombosis, acute coronary artery disease, ischemia/reperfusion injury and infectious diseases such as bacterial sepsis. Understanding the role of coagulation in inflammation and the impact of inflammation on coagulation will introduce new perspectives to improve diagnostics and therapies for both disease states.

The plasma contact system is a pro-inflammatory and procoagulant protease cascade that is initiated by factor XII (FXII), in a reaction involving high molecular weight kininogen (HK) and plasma kallikrein (PK).[1] Upon contact with anionic surfaces, a conformational change occurs in zymogen FXII resulting in active FXII (FXIIa). FXIIa initiates the intrinsic pathway of coagulation via activation of the FXII substrate factor XI (FXI), and the bradykinin (BK) producing kallikrein-kinin system via plasma kallikrein.[2] Binding of BK to B2 receptor (B2R) activates intracellular signaling pathways that increase vascular permeability. C1 esterase inhibitor (C1INH) is the major plasma inhibitor of activated FXII and PK. Factor XII contact activation triggers coagulation in the diagnostic clotting assay 'activated partial thromboplastin time' (aPTT), however, deficiency in the protease is not associated with hemostatic abnormalities in humans and mice. Challenging the concept of the coagulation balance, thrombus formation is defective in FXII deficient (*F12*$^{-/-}$) mice.[3] Consistently, pharmacological inhibition of FXIIa-driven coagulation provides thromboprotection in large animals and humans[4] without an increase in therapy-associated bleeding. The biochemistry of the contact system is well-described but its biological functions, however, have just emerged within the last years. This short review summarized major findings in the contact system area of the last year. A more detailed overview is given in the latest reviews.[5,6]

Current state of the art

For decades, blood platelets have been known to activate FXII-driven coagulation the underlying mechanisms, however, have remained unknown. Until recently polyphosphate (polyP) was identified as a novel platelet derived FXII activator with critical implications for platelet-driven thrombosis and inflammatory reactions *in vivo*.[7,8] PolyP is an inorganic polymer of orthophosphate units linked by phosphoanhydride bonds. The polymer is ubiquitously found in all living cells and varies in chain length from just a few to several thousand phosphate units. In mamls, polyP stimulates an array of procoagulant mechanisms and drives fibrin formation by activation of the FXII-driven contact system.[9] Experimental animal models suggest a role of polyP in platelet-driven arterial and venous thrombosis. However, the potential therapeutic implications of interference with this substance have remained to be elucidated.

We have recently developed specific inhibitors of polyP and shown that this strategy confers thromboprotection in a FXII-dependent manner.[10] Exopolyphosphatase (PPX) is an intracellular enzyme that specifically hydrolyses polyP. To target the FXII activator polyP, we developed recombinant E. coli PPX

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mutants that specifically neutralized circulating polyP. Interference with polyP abolished platelet-driven fibrin formation and thrombus formation in human blood. Targeting polyP interferes with FXII activation and selectively reduces the activated FXII-driven 'intrinsic' pathway of coagulation. Neutralizing blood-borne polyP in mice potently interferes with experimental arterial thrombosis and provides protection from venous thromboembolism in a pulmonary embolism model. Despite its potent antithrombotic activity, specific ablation of circulating polyP, similar to targeting FXII, does not prolong the bleeding time in mice and does not cause excess blood loss from injury sites. The study provides mechanistic insight as to the procoagulant properties of polyP and introduces ways to specifically interfere with its function. Disruption of blood-borne polyP proved to not interfere with hemostasis and may thus pave the way for the development of novel anticoagulation approaches with an improved benefit-to-risk profile in comparison with currently available agents.

Recently elegant imaging studies have visualized platelet polyP on living cells.[11] Platelets contain two pools of polyP: short polymers of a chain length ranging between 60-100 that are soluble and released into the supernatant. The majority of platelet polyP, however, is long chain molecules of chain length >500 that are bound to calcium ions and are not soluble in plasma. Calcium-polyP forms procoagulant nanoparticles of 100-200nm diameter that are retained on the platelet surface and readily activate FXII. Additionally, a promising assay to analyze polyP in human samples has been recently established.[12] A flow cytometry (FACS)-based assay using a recombinant polyP specific probe (PPX_Δ12) quantifies polyP exposed on cells such as activated platelets. Using this FACS-based system, the authors showed that platelets expose polyP aggregates e.g. nanoparticles on their surface that function as powerful activator of coagulation.

In addition to its procoagulant activities the contact system is a powerful pro-inflammatory pathway. The life-threatening swelling disorder hereditary angioedema (HAE) develops in individuals who are deficient in functional C1 esterase-

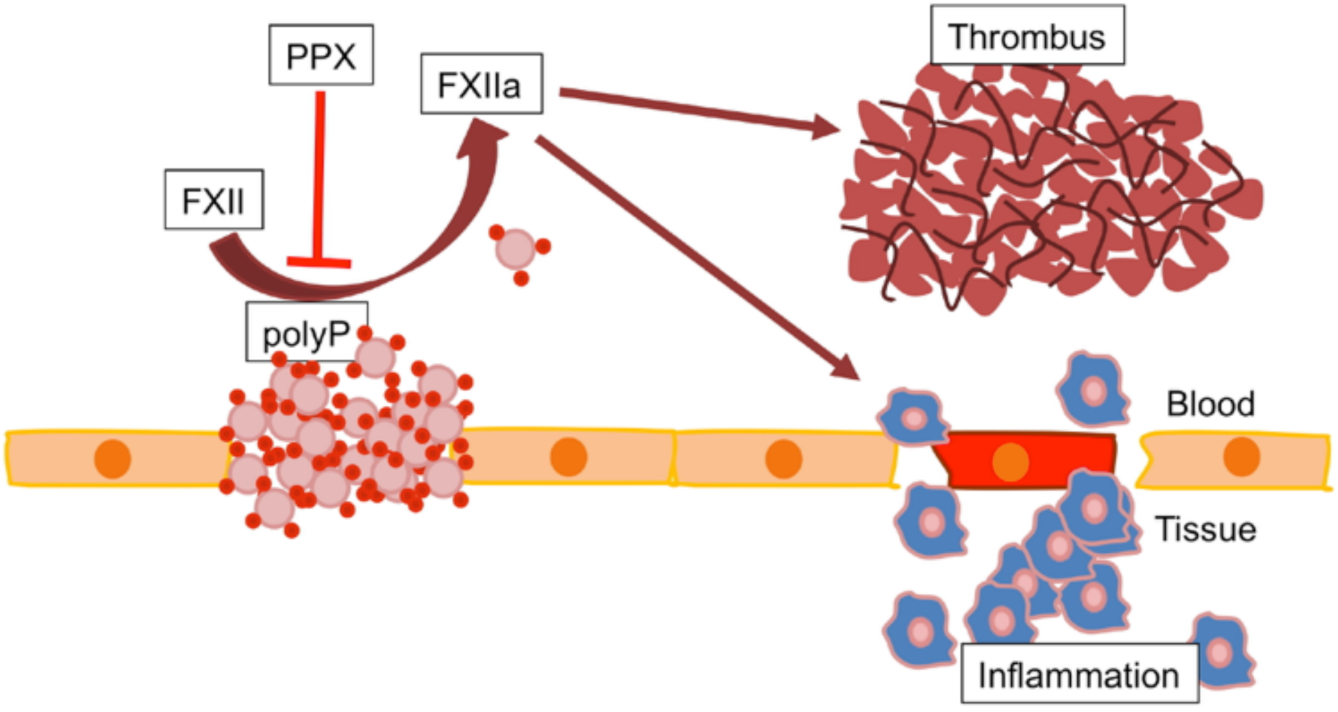

Figure 1. The polyphosphate-factor XII pathway in thrombosis and inflammation. Activated procoagulant platelets release the inorganic polymer polyphosphate (polyP) that is retained as calcium ion-rich nanoparticles at the cell surface. Binding of factor XII (FXII) zymogen to platelet polyphosphate induces contact activation and produces the active protease (FXIIa). Activated FXII drives thrombosis and vascular inflammation via the intrinsic coagulation pathway or kallikrein kinin system, respectively. The exopolyphosphatase PPX specifically degrades polyP and thus interferes with thrombosis and inflammation in an FXII-dependent manner.

inhibitor (C1INH; HAE types I and II). In addition to these two classic HAE types a third variant, HAE type III, exists in patients that have a completely normal C1INH but similarly suffer from edema attacks. The pathophysiology of HAE type III has remained unresolved, which limited therapy for this condition. GWAS studies have associated specific FXII point-mutations (Thr309Lys, Thr309Arg) with HAE type III. Using mouse edema models and patient materials, we have recently identified the mechanisms of HAE type III.[13] FXII-derived from HAE type III patients is defective in a mucin-type Thr309-linked glycosylation. Mutant FXII displays aberrant activation, which leads to excessive production of bradykinin via the kallikrein-kinin pathway. Intra-vital laser-scanning microscopy shows increased contact-driven microvascular leakage in both *F12*$^{-/-}$ mice reconstituted with recombinant FXII mutants and in humanized HAE type III mouse models with inducible liver-specific expression of Thr309Lys-mutated FXII. A FXII-neutralizing antibody, but not C1-esterase inhibitor that interferes with edema in HAE types I and II, abolished bradykinin generation in HAE type III patient plasma and blunted edema in HAE type III mice. The study characterized the mechanism of HAE type III and established FXII inhibition as a novel therapeutic strategy to interfere with excessive vascular leakage in HAE and potentially, other causes of edema and anaphylaxis.[14]

Recently a natural trigger for FXII activation, which causes uncontrolled bradykinin production in patients with HAE type III has been identified.[15] Recombinant variants of FXII revealed, that the HAE type III-associated mutations collectively introduce new sites that are sensitive to enzymatic cleavage by the protease plasmin. FXII mutants found in HAE type III rapidly activate following cleavage by plasmin, escape inhibition through C1 esterase inhibitor, and drive excessive bradykinin formation indicating that plasmin modulates disease activity in patients with HAE type III. These findings indicate a new pathway for bradykinin formation in patients with HAE in which FXII is cleaved and activated by plasmin. Taken together, the contact system has emerged as a promising drug target for interference with inflammation and coagulation *in vivo* (Figure 1). Understanding the interplay of coagulation and inflammation triggered by the FXII-driven contact system will open perspectives for safe anticoagulant drugs with additional anti-inflammatory activities.

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