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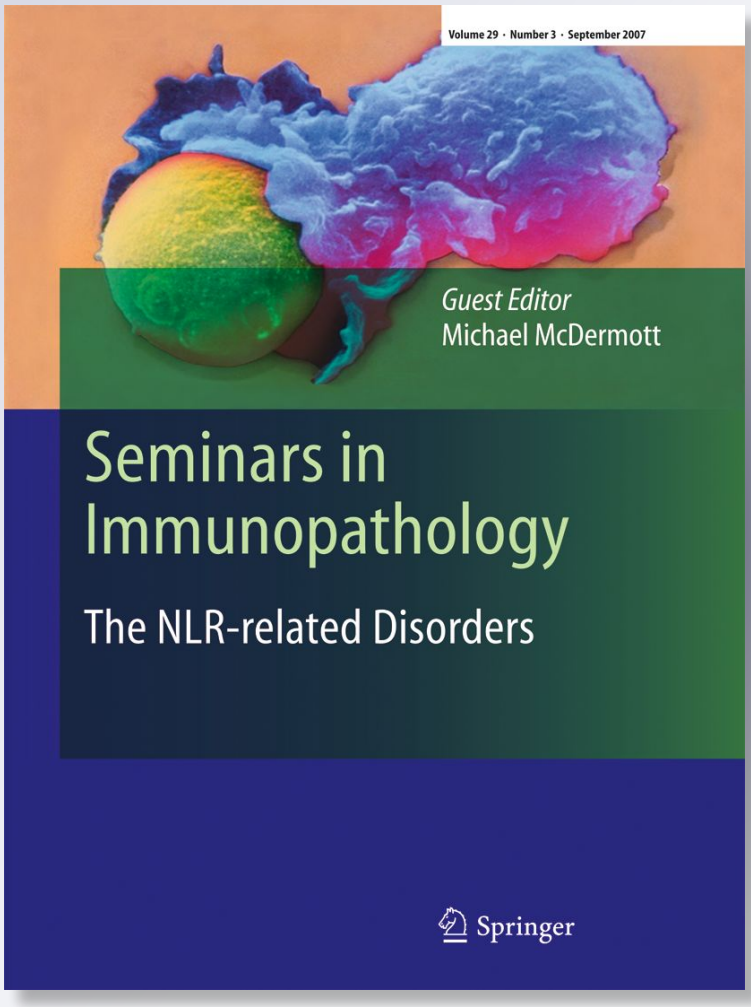

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REVIEW

The procoagulant and proinflammatory plasma contact system

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Abstract The contact system is a plasma protease cascade that is initiated by coagulation factor XII activation on cardiovascular cells. The system starts procoagulant and proinflammatory reactions, via the intrinsic pathway of coagulation or the kallikrein–kinin system, respectively. The biochemistry of the contact system in vitro is well understood, however, its in vivo functions are just beginning to emerge. Data obtained in genetically engineered mice have revealed an essential function of the contact system for thrombus formation. Severe deficiency in contact system proteases impairs thrombus formation but does not reduce the hemostatic capacity of affected individuals. The system is activated by an inorganic polymer, polyphosphate that is released from activated platelets. Excessive inherited activation of the contact system causes a life-threatening swelling disorder, hereditary angioedema. Activation of the contact system by pathogens contributes to leakage in bacterial infections. Mast-cell-derived heparin triggers contact-system-mediated edema formation with implications for allergic disease states. Here we present an overview about the plasma contact system in occlusive and inflammatory disease and its contribution to health and pathology.

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Components of the plasma contact system

Blood coagulation is essential to maintain the integrity of a closed circulatory system (hemostasis), but may also contribute to thromboembolic occlusions of the vessel lumen, which obstruct the blood flow resulting in tissue damage (thrombosis). Thrombosis may occur in the venous or arterial circulation causing pulmonary embolism or myocardial infarction and stroke, respectively, collectively the most common causes of death in the developed world [1]. Injury to a blood vessel triggers activation of blood platelets and the plasma coagulation system, leading to formation of a blood clot consisting of platelets and fibrin. Formation of fibrin is mediated by a group of tightly regulated plasma proteases and cofactors, the blood coagulation cascade. In the original waterfall models fibrin formation is initiated by two distinct pathways, triggered by exposure of blood to a damaged vessel wall (extrinsic) or to blood-borne (intrinsic) factors [2]. The intrinsic pathway of coagulation is initiated by factor XII (FXII, Hageman factor), in a reaction involving high molecular weight kininogen (HK) and plasma kallikrein (PK), collectively referred to as plasma contact system. Contact to negatively charged surfaces induces a conformational change in FXII zymogen, resulting in a small amount of active FXII (FXIIa). FXIIa cleaves PK to generate active kallikrein, which in turn reciprocally activates additional FXII [3]. FXIIa initiates fibrin formation through its substrate factor XI (FXI) [4] and also triggers liberation of the inflammatory mediator bradykinin (BK) by kallikrein-mediated HK cleavage [5]. Contact system proteins assemble on heparan-type glycosaminoglycan chains [6, 7] of

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membrane-associated proteoglycans in cells of the cardiovascular system [8]. BK is the ligand of the kinin B2 receptor (B2R), a G-protein-coupled receptor present on endothelial cells. The binding of BK to its cognate B2R activates various intracellular signaling pathways that dilate pressure-regulating vessels, induce chemotaxis of neutrophils, and increase vascular permeability and fluid efflux [9]. BK induces vasodilatation by stimulating cellular production of nitric oxide (NO) and induces symptoms of inflammation such as swelling, redness, and pain. In plasma, BK is converted to des-Arg9-BK, which binds the kinin B1 receptor (B1R). This receptor is in inflamed tissues and contributes to renal fibrosis, alterations in blood–brain barrier permeability [10], and regulation of adaptive immunity [9, 11]. BK has a very short half-life in vivo (a matter of seconds) and is degraded by kininases, including angiotensin-converting enzyme (ACE) [6]. The prolonged half-life of the vasodilator BK contributes to the blood-pressure-lowering effects of ACE inhibitor treatment. Conversely, dry cough and respiratory reactions, known adverse effects of ACE inhibitors, are attributed to elevated BK levels. A scheme of the FXII-triggered contact activation system is given in Fig. 1.

FXII activation by artificial polyanions such as glass initiates fibrin formation in vitro and FXII-contact activation by kaolin (a silicate) or ellagic acid provides the molecular basis for one of the most commonly used diagnostic clotting tests, the activated partial thromboplastin time (aPTT). The aPTT is widely used in clinical clotting analyses. Despite its critical function for coagulation *in vitro*, FXII-driven contact activation was believed to have no function in vivo. The premise is based on the well-known observation that FXII deficiency is not associated with any hemorrhagic disorder in FXII humans or animals. Individuals with severe FXII deficiency have a prolonged aPTT, but do not suffer from abnormal spontaneous or injury-related bleeding [12]. Indeed, numerous patients lacking FXII have undergone surgery with no apparent excess bleeding, despite abnormal results on in vitro clotting assays [13]. Since deficiency in FXII is not associated with increased spontaneous or injury-related bleeding tendency, FXII was considered to play no role in blood coagulation in vivo, and subsequent revisions of the coagulation cascade usually exclude FXII [14]. The absence of a bleeding phenotype in FXII deficiency, in contrast to deficiencies of other components of the cascade such as factor VII, tissue factor (TF), and factor IX (deficiency causes the bleeding disorder Hemophilia B), has lead to the reasonable hypothesis that fibrin formation in vivo is initiated largely, if not exclusively, through the extrinsic pathway of coagulation by factor VIIa/TF [15]. The factor VIIa/TF-driven model for hemostasis is supported by the observations that FXI, a major substrate for activated FXII (FXIIa) during contact-initiated clotting, can be activated by thrombin independently of FXII. The FXII-independent "revised model of coagulation" had been widely accepted [14].

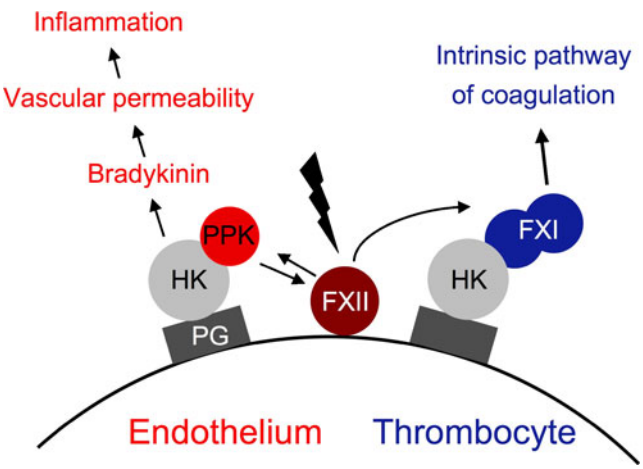

Fig. 1 Coagulation factor-XII-driven contact activation system. Polyanionic surfaces such as polyphosphates (see below) trigger coagulation factor XII (*FXII*) activation on endothelial cells and thrombocytes initiating procoagulant and proinflammatory cascades. FXII activates its substrate factor XI (*FXI*), driving fibrin formation by the intrinsic pathway of coagulation. Alternatively, FXII initiates bradykinin generation by plasma kallikrein-mediated cleavage of high molecular weight kininogen (*HK*) on endothelial cell surface proteoglycans (*PG*). The peptide hormone BK is an inflammatory mediator and increases vascular permeability

FXII in thrombus formation

FXII-deficient ($FXII^{-/-}$) mice were generated and phenotyped to study the function of coagulation FXII in vivo [16]. Similar to FXII-deficient humans, $FXII^{-/-}$ mice have a normal hemostatic capacity as assessed by a tail-bleeding assay [17]. Completely unexpected, intravital fluorescence microscopy and blood flow measurements in three distinct arterial beds revealed a severe defect in FXII-deficient mice in thrombus formation induced by different methods of injuries [17]. The data with FXII null mice challenge the "revised model of coagulation" and demonstrate a crucial role of FXII for fibrin formation in vivo. Reconstitution of FXII null mice with human FXII restored the prolonged aPTT found in untreated animals and fully restored the capacity of infused animals for thrombus formation. The reconstitution experiments suggest that FXII operates similarly in mice and humans. Indeed, the contact system is highly conserved among mammalian species [18]. Thrombus formation in FXII heterozygous mice—having 50% of normal plasma levels—was similar to wild-type control mice (100% FXII), indicating that half of normal plasma concentration is sufficient for vessel occlusive clot formation. Vice versa, drugs that target FXII activity need to substantially reduce activity of the protease to provide thromboprotection. The essential role of FXII for thrombus formation has implications for thrombo-

embolic disease. FXII-gene-deficient mice are protected from the effects of cerebral ischemia in an experimental stroke model [19]. This protective effect is related to reduced fibrin formation in the microvasculature of the ischemic tissue. Mice lacking FXI are similarly protected from vessel occlusive fibrin formation, suggesting that FXII impacts only on pathologic clotting via the intrinsic pathway. Despite the striking protective effect in these models, FXII-deficient mice, like their human counterparts, do not have spontaneous or injury-related hemorrhage. Pharmacologic inhibition of FXII activation using the peptide-based inhibitor PCK in wild-type mice also provides protection from cerebral ischemia, without causing excessive bleeding at a surgical injury site. Consistently, a recombinant infestin-4-based inhibitor that specifically targets FXII activity provided protection from cerebral ischemia in experimental stroke models albeit did not affect the hemostatic capacity in inhibitor-treated mice [20]. These results demonstrate that FXII-driven fibrin formation is specifically important for pathologic thrombus formation but has no function for fibrin formation during normal hemostasis. This raises the exciting possibility that targeting FXII may offer a strategy for prevention or treatment of pathological thrombosis that is not associated with the high rate of hemorrhage that accompanies currently used anticoagulants. Furthermore, given the results found in mouse models, the concept that pathologic thrombus formation represents disequilibrium of the processes that normally produce a clot at a site of injury likely needs revision (Fig. 2).

The importance of the contact system for thrombosis is not restricted to animal models. Proteins of the contact system are highly conserved between mice and humans and results from animal models can easily be transferred to human disease states, e.g., FXI appears to participate to thrombosis in humans similarly to its role in mouse models. Together, the new data from contact-system-deficient mice and patients challenge the concept that pathological thrombus formation represents a dysregulation of normal hemostatic mechanisms and suggest that the dogma of the coagulation balance is in need for revision [21].

In test tubes in plasma FXI can be activated both by FXIIa and by thrombin according to the classical or revised model of coagulation, respectively. A comparison of mice with deficiency in FXI, FXII, or combined deficiency in the two clotting factors did not reveal additional thromboprotection in FXI/FXII-double-deficient mice in a model of lethal pulmonary embolism [22]. The data indicate that FXII is predominantly if not exclusively operating via its substrate FXI in pathological thrombus-forming mechanisms in mice. Consistently, antibodies that specifically block FXIIa-mediated FXI activation interfere with thrombus formation in baboons [23]. However, FXI may also be activated by thrombin independently of FXII [24]. The latter study strongly supports the existence of FXI feedback activation in vivo [25]. The relative importance of FXIIa versus feedback activation for generating active FXI in vivo are not completely understood and may have different importance depending on injury type, vascular bed, and site of activation.

FXII deficiency and thrombosis in humans

In contrast to the conclusive data in genetically altered mice, there is a lack of clinical studies that systematically

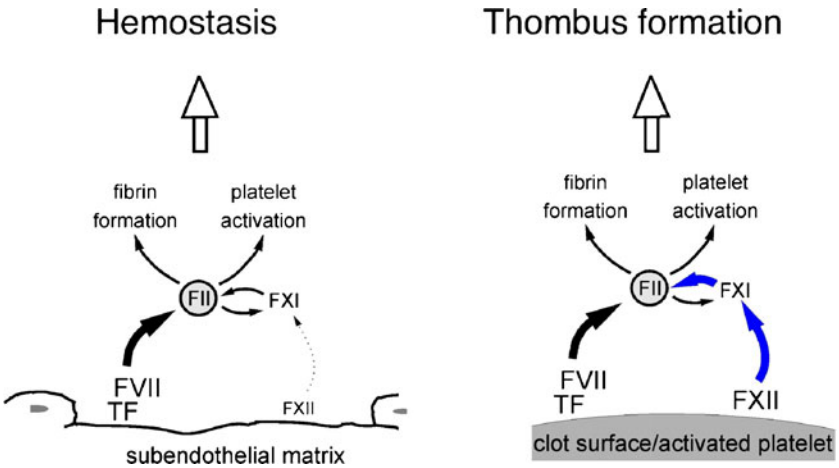

Fig. 2 A revised model of thrombus formation. (*left*) Thrombin (*FII*) formation at sites of lesions is predominantly due to tissue factor (*TF*) exposed in the subendothelial matrix. TF in complex with factor VII (*FVII*) initiates thrombin formation, which promotes fibrin formation and platelet activation. The contribution of factor XII (*FXII*) for hemostasis is minor. (*right*) Additional fibrin-forming activity is necessary to form a thrombus. FXII activity in the growing thrombus contributes to thrombin generation and additional platelet activation, propagating thrombus growth. Accordingly, FXII- as well as factor XI (*FXI*)-deficiency severely impairs thrombus formation but have no function for hemostasis (figure adapted with minor modifications from [17])

compare the incidence or severity of thromboembolic events in humans with severe FXII deficiency and normal individuals. However, there is a long history of case reports suggesting that FXII deficiency may actually predispose to thrombosis, going back to the death of the original FXII-deficient patient John Hageman, who died from pulmonary embolism [26]. Challenging these anecdotic reports that have suggested FXII deficiency to be a prothrombotic risk factor careful reanalysis showed that thrombosis in FXII-deficient patients is related to other thrombotic risk factors than FXII deficiency [27]. Indeed, large patient studies in the Netherlands and Switzerland clearly demonstrated no correlation between FXII deficiency and increased thrombotic risk [28, 29]. Since severe FXI is common in Jews particularly of Ashkenazi origin, several studies have analyzed thrombotic events in these individuals [30]. A recent study has analyzed incidence of ischemic stroke in Jews with severe FXI deficiency. Similarly to FXI-null mice [19], FXI-deficient humans are protected from cerebral ischemia [31] supporting the decisive role of the intrinsic pathway for arterial thrombosis in humans. Thromboprotection in FXI-defective patients is not restricted to arterial beds. Deficiency in the clotting factor provides protection from deep vein thrombosis [32]. In contrast to FXI-deficient mice humans with deficiency suffer from increased bleeding (Hemophilia C). Bleeding in FXI-deficient patients is usually mild as compared to patients lacking factors VIII or IX (Hemophilia A and B) and mostly limited to tissues with high fibrinolytic activity.

Although clinical studies have shown that incidence of thrombotic events is similar in FXII-deficient and normal individuals, the detailed association of FXII deficiency and risk for thrombosis is probably more complex. Defective thrombus stability in $FXII^{-/-}$ mice revealed by intravital microscopy [17], reports of pulmonary emboli in humans with congenital FXII deficiency [33], and reduced clot firmness in FXIIa-inhibited blood as shown by thromboelastography in mice [22] and humans [34] have raised an intriguing hypothesis. Severe FXII deficiency interferes with thrombus propagation apart from the vessel wall and provides thromboprotection. However, severe FXII deficiency may also have adverse effects. It is tempting to speculate that deficiency in FXII might have increased the risk for Mr. Hageman for pulmonary embolism due to defective fibrin formation via this factor. Although more studies are necessary the data suggest that FXII deficiency could be a risk factor for embolic disease such as pulmonary embolism, i.e., under circumstances, when a thrombus has developed by FXII-independent stimuli, especially when thrombosis is driven by massive tissue factor exposure, offering a new rational for the historical case of John Hageman. FXII levels and overall mortality and death from cardiovascular disease have a peculiar bell-shaped correlation; mortality of patients with severe FXII deficiency was similar to that of individuals having 100% FXII plasma levels [35] indicating that thromboprotection from arterial thrombosis is counterbalanced by other mechanisms in FXII-deficient individuals.

Hereditary angioedema

The contact system has the capacity of activating the classical pathway of the complement system [36] and simultaneous activation of the contact and complement system may often occur under pathological conditions. Hereditary angioedema (HAE [MIM #106100]) is a life-threatening swelling disorder that develops in individuals, who are deficient in C1-esterase inhibitor (C1INH, HAE type I), or have a dysfunctional C1INH protein (HAE type II). C1INH is a plasma protein and belongs to the serpin family of serine protease inhibitors. C1INH is also the major inhibitor of the classical complement pathway proteases C1r and C1s and regulates generation of the inflammatory mediator BK by the plasma contact system [5]. Deficiency in functional C1INH facilitates excessive activation of the complement and contact system cascades and the development of edema in HAE type I and II patients [37, 38]. In addition to these two classical HAE types, a third variant exists that almost exclusively affects women. HAE type III patients have normal biochemical C1INH functions and plasma concentration but similarly to other patients suffering from edema [39]. Clinically, all types of HAE are characterized by recurrent episodes of acute swelling involving the skin or the oropharyngeal, laryngeal, or gastrointestinal mucosa. The pathophysiology of increased vascular permeability in HAE has remained controversial. Elegant studies with genetically modified mice demonstrated that edema formation in C1INH-dependent HAE forms is due to pathological contact system activation [40]. Targeted ablation of C1INH increased FXII and PK activity and resulted in excessive PK-mediated release of BK from its inactive precursor, HK. BK activates intracellular signaling cascades by binding to B2R, which increases vascular permeability in humans [41] and mice [40]. Comprehensive analysis of BK have shown increased levels of the mediators in acute swelling attacks strongly suggesting that increased BK activity mediated elevated vascular leakage [42]. Hence, HAE types I and II are treated by infusion of plasmatic or recombinant C1INH [43] or by B2R antagonists (Icatibant) [44]. Alternatively, recombinant PK inhibitors (Ecallantide) may be used to interfere with acute swelling attacks in HAE patients [45].

In contrast to C1INH-dependent HAE forms, the pathomechanism and therapy of HAE type III has remained

enigmatic. Using genome-wide linkage analyses in families from France, Switzerland, and Germany, HAE type III was shown to be an autosomal dominant disease and association with a single missense mutation (c.1032CrA) in the gene of coagulation FXII [46]. A later study involving other families confirmed the findings of Cichon and coworkers and showed HAE type III to be associated with a different mutation that affects the same nucleotide of the FXII gene, c.1032CrG [47]. Both point mutations translate into amino acid exchanges Thr328Lys and Thr328Arg, respectively, on the protein level. In plasma of HAE type III patient, the enzymatic activity of FXII is elevated as compared to healthy controls [46]. Of note, the aPTT assay results in normal values in HAE type III patients and fails to detect affected individuals. The aPTT assay starts fibrin production by preactivated FXII. FXII is activated by exposure to strong contact activators such as kaolin for about 120–180 s. This time period is sufficient for generating maximal FXII activity independent on presence or absence of the endogenous inhibitor C1INH or FXII-activating conformations (forms of FXII promoting its active potential) such as found in the FXII-Thr328 mutants [48].

Estrogens regulate FXII expression [49], which might be responsible for the women's specificity of HAE type III [50]. However, FXII plasma levels in HAE type III patients are in the normal range [46], suggesting that yet unknown mechanism trigger edema predominantly in women. Diagnosis of HAE is difficult as the clinical symptoms are highly variable regarding localization, frequency, triggering factors, and severity of swellings. In contrast to HAE types I and II that are diagnosed by analysis of C1INH plasma levels and function, diagnosis for HAE type III is elaborate and needs FXII gene sequencing that is available in only few diagnostic laboratories. The clinical symptoms suggest that edema in HAE type III is due to aberrant BK formation; however, other FXII-driven pathways such as the intrinsic pathway of coagulation, the complement system, or the fibrinolytic system may contribute [51]. HAE patients experience recurrent attacks of swelling, but the stimuli that trigger these periodic episodes of excessive vascular leakage are poorly defined [37, 38]. BK is generated in allergic disease and contributes to increased vascular permeability [44, 52, 53]. In vitro, the mast cell-derived polysaccharide, heparin liberates BK by triggering contact system activation [54–56]. The data obtained in plasma samples suggest a role of heparin for triggering swelling episodes in HAE patients in vivo.

Mast-cell-driven edema formation

Mast cells are highly effective sentinel cells that are found close to blood vessels and are especially common at sites of potential infections, such as the skin, airways, and gastrointestinal tract. Mast cells have been most extensively studied in their traditional role as an early effector cell of allergic disease; however, these cells also participate actively in innate immune responses to many pathogens [57]. A hallmark of mast cell activity in host defense and allergic reactions is increase in vascular permeability. It was believed that mast-cell-triggered leakage is predominantly if not exclusively mediated by histamine release and targeting histamine signaling is widely used therapeutically to treat edema formation associated with aberrant mast cell activity [58]. In addition to histamine, mast cell secretory granules also contain highly sulfated polysaccharides, with heparin as a major constituent. The glycosaminoglycan, which is synthesized exclusively by mast cells, has a crucial role as a depot for various mediators and for the morphology of the granules. Targeted inactivation of a specific sulfotransferase (*N*-deacetylase *N*-sulfotransferase-2, NDST-2, an enzyme that contributes to modify the polysaccharide chain) largely diminishes granule number and contents [59, 60]. Addition of mast cell derived heparin-initiated BK formation in plasma in an FXII-dependent manner. Heparin as low as 4 μg/ml was sufficient to initiate the FXII/PK cascade resulting in HK procession. Under these conditions the FXII substrate FXI was not activated. Similarly to heparin, misfolded protein aggregates that were previously shown to have contact system activating capacity specifically initiate the kallikrein–kinin system and do not trigger activation of the intrinsic pathway of coagulation. The detailed mechanism for selective activation of the FXIIa substrate PK over FXI is not entirely clear but may reflect a higher plasma concentration of PK *versus* FXI and higher specificities of heparin-dependent plasma inhibitors such as antithrombin III (AT III) for activated FXI *versus* PK.

Consistent with ex vivo data [56], topical heparin application increased vascular permeability in an FXII-dependent manner in mice. Intravital confocal laser scanning microscopy and classical tracer extravasation experiments (Miles edema model) identified BK as the active mediator for increased leakage in heparin-driven plasma extravasation in skin microvessels [61]. Comparisons of wild-type mice with animals having defective heparin-induced BK effects show that heparin-driven BK formation accounts for a large portion (about 50%) of mast-cell-evoked alterations of vascular permeability. Consistently, small molecule inhibitors of FXII or B2R antagonists interfere with mast-cell-triggered leakage. Based on the experimental data, targeting heparin-initiated BK formation may represent a promising strategy to protect against defective barrier function attributed to mast cell activation in a broad variety of diseases. Indeed, FXII gets activated on particulate matter that is a component of air pollution

[62]. BK formed by the FXII-driven contact pathway may contribute to intestinal pulmonary edema in allergic responses to air pollution. A similar mechanism contributes to bacterial infections. Contact system proteins assembled on the surface of *Escherichia coli* and *Salmonella* through their specific interactions with fibrous bacterial surface proteins, curli and fimbriae, and induce proinflammatory reactions via BK release [63]. Pharmacological inhibitors of contact system-driven BK formation interfere with *Salmonella*-mediated pneumonia and pulmonary leakage in rodent models [64]. BK activity generated by a FXII-independent mechanism involving carboanhydrase increases retinal permeability [65] with possible implications for pathology and therapy of proliferative diabetic retinopathy and diabetic macular edema. Cumulatively, the data highlight the role of BK for increasing vascular permeability in inflammatory and infectious diseases states.

Hereditary angioedema is characterized by recurrent swelling attacks. The underlying disease mechanism is a deficiency in functional C1INH [66] or a gain of function mutation in FXII [46] resulting in increased susceptibility for contact-system-driven BK formation. In HAE patients, poorly defined stimuli initiate the contact-system-driven excessive BK formation that increases vascular leakage in patients [38]. As heparin was identified as potent activator of contact-system-mediated BK generation in vitro and in vivo, we reasoned testing allergen-activated mast cells that release heparin from endogenous granules as potential initiator of swelling attacks in HAE. We targeted deleted C1INH (the major inhibitor for FXII) in mice to generate an animal model for hereditary angioedema. C1INH null mice were highly susceptible for activated mast-cell-triggered vascular leakage and IgE challenge readily initiated angioedema in these animals. Following IgE-mediated mast cell activation, leakage occurred faster and was more pronounced in C1INH null mice as compared to wild-type controls. $FXII^{-/-}$ and $B2R^{-/-}$ mice were partially protected (about 50% less edema) from allergen-activated mast-cell-driven edema indicating a significant role of the kallikrein–kinin system in mast-cell-triggered alterations of membrane stability. Supporting a critical function of mast-cell-released heparin for activating the contact system under pathological conditions systemic mast cell activation significantly contributes to anaphylactic reaction and drop in blood pressure. B2R antagonists largely block these adverse effects and might be beneficial for treating individuals suffering from allergic hypotonic reactions or even shocks. In a substantial portion of HAE patients, onset of swelling attacks was triggered by allergic reactions to food, drugs, or insect stings [61]. The data in experimental mouse systems and patients identify subclinical pathological mast cell activation as potential initiator of swelling episodes in HAE patients. In these individuals the contact system is hyper-activateable (either due to deficiency in C1INH function or due to activating FXII mutations) and FXII-contact-activating agents at concentrations that are not sufficient to start the reaction cascade in healthy individuals initiate the BK-forming reaction.

Contaminated heparin

For decades heparin is widely used as an anticoagulant drug. The polysaccharide prevents the formation and extension of blood clots in the circulatory system via increasing AT III activity. Starting November 2007, there was a dramatic increase in heparin-induced adverse reactions such as lethal acute hypersensitivity reactions in the USA and Germany in patients intravenously receiving commercial heparin of specific lots from a single manufacturer (http://www.fda.gov/cder/drug/infopage/heparin/adverse_events.htm). Comprehensive analyses identified a non-naturally contaminant occurring in suspect preparations of heparin that was characterized as over-sulfated chondroitin sulfate (OSCS) [67]. OSCS-contaminated heparin has a greatly increased potency for activating FXII and triggering PK-mediated BK formation in human plasma and in a model of experimental hypotonic shock in vivo [68]. These catastrophic reactions in patients are reminiscent of experimental shock models induced by stimulated BK formation in pigs. Infusion of the highly negatively charged polysaccharide dextran sulfate (a strong non-naturally occurring contact system activator [69]) induced transient systemic hypotension and Icatibant (previously known as Hoe-140, a B2R antagonist) blocked the drop in blood pressure [70]. These data support a role of BK in immediate adverse reactions triggered by negatively charged polysaccharides. BK-forming activity seems to be dependent on negative charge density of the polysaccharide rather than on a defined structure. Indeed, potency of FXII-driven contact activation in a reconstituted system decreased from dextran sulfate and OSCS (with an average four sulfate residues per disaccharide) [69, 71], to mast cell heparin (with an average of ≈2.7 sulfate residues per disaccharide) [56], whereas heparan sulfate (with an average of ≈1 sulfate residue per disaccharide) was inactive. The potency to activate the plasma contact system also greatly varies among diverse heparin preparations [54, 55] reflecting differences in purification procedures, sources of the polysaccharides, and experimental settings. Some purification procedures of clinically used heparins fragment the polysaccharide backbone, modify its structure [72] and modulate the activity for inducing BK formation by the contact system. Of note, although intravenous heparin infusion may trigger BK generation, infusion of the polysaccharide even at high concentrations in a bolus does

not induce drop in blood pressure nor cause edema. BK that is generated in venous vessels is rapidly and almost completely degraded by ACE and other kininases that are abundantly expressed in lung microvessels before reaching precapillary vascular beds, which regulate the blood pressure.

Charge-dependent contact system activation is not restricted to polysaccharides. It is known for decades that FXII binds to insoluble collagen, enhancing coagulation [73]. The interaction is dependent on a repetitious presentation of negative charges in the native collagen fibril [73]. However, later reports argued against a direct activation of FXII by collagen. Recent studies have re-evaluated the importance of collagen for FXII activation [74]. FXII was shown to bind to collagen fibrils of various origins. When added to plasma (equine) type I collagen promoted thrombin formation and plasma clotting in an FXII-dependent manner. FXII activity is critical for collagen-stimulated thrombus formation in flow chambers [75]. Additionally, collagens may stimulate the contact system indirectly [22].

Activation of the FXII-driven intrinsic pathway of coagulation

Platelet activation has been linked to the intrinsic pathway [76] for more than half a century. Activated platelets promote fibrin formation in an FXII-dependent manner in vitro [77]. However, based on normal hemostasis in FXII-deficient individuals, platelet-driven FXII activation was neglected. Cumulatively, the classical data suggest that FXII is activated on or by procoagulant platelets. The existence and identity of the endogenous activator of FXII in vivo has puzzled investigators for decades. Upon activation platelets release from their dense granules various compounds such as serotonin, Ca^{2+}, ADP, and ATP, as well as large amounts of polyphosphates [78]. Polyphosphates (polyP) are inorganic, linear polymers of orthophosphate units linked by phosphoanhydride bonds. PolyP is abundantly found in nature and is highly conserved throughout evolution. The polymer is found from bacteria to mammalian cells. Synthetic polyP is used as water softener in technical processes and has been shown to be a potent modulator of plasma clotting, affecting the intrinsic pathway, the fibrinolytic system, factor V activation, and the fibrin structure [79]. As FXII is readily activated by contact to polyanionic surfaces, we wondered whether activated platelets would secret a natural kaolin analogue and systematically screened for activated platelet released polyanions [22]. Activation of human and mouse platelets with various platelet activators such as thrombin receptor activating peptide 6 (Trap6) and thrombin released an inorganic polymer, polyphosphate, that was purified from the platelet supernatant using an anion-exchanger-based method. A toluidine blue and a more sensitive DAPI staining (Fig. 3 A and B) showed platelet polyP that were separated on agarose gels. Notably, polyP is not a homogenous substance but the polymers differ in chain length resulting in a smeary signal in the agarose matrix.

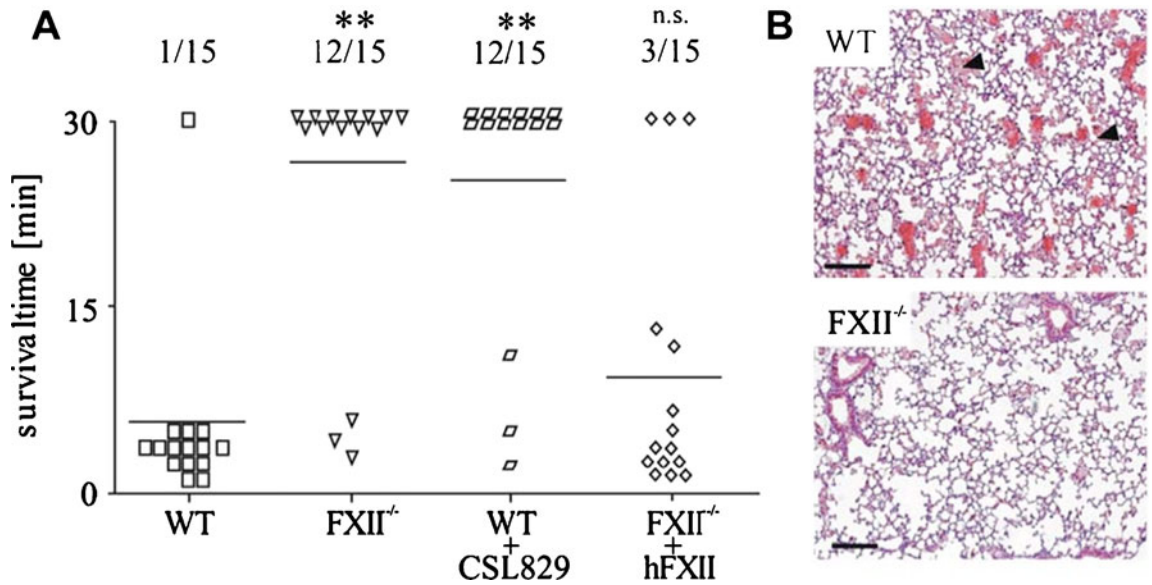

Fig. 3 Polyphosphates initiate thrombosis in a FXII-dependent manner. Venous application of polyP leads to FXII-dependent lethal pulmonary embolism. (*A*) PolyP were intravenously applied in wild-type (*WT*) and FXII-deficient mice ($FXII^{-/-}$), in FXII-inhibitor-treated wild-type (*WT + CSL829*) and $FXII^{-/-}$ mice that were reconstituted with human FXII ($FXII^{-/-}$ + *hFXII*) and survival of the animals was documented for 30 min. (*B*) Lung histology showed clots in WT animals, whereas no thrombi were found in $FXII^{-/-}$ mice (figure modified from [22])

Incubation of isolated polyP with phosphatase (an enzyme that cleaves phosphoester bonds) degraded the polymer and fully abolished the polyP signal. How long are platelet polyPs? The endogenous ^{31}P signal in the polymer chain differs whether the phosphorous nucleus is at the edge or within the polymer chain. ^{31}P-NMR spectroscopy revealed an average chain length of platelet polyP of about 80 orthophosphate subunits. What is the biological function of platelet polyP? PolyP potently initiated FXII activation in a time- and dose-dependent manner highly similarly to kaolin (a strong FXII activator). Endogenous thrombin potential analyses and Western blotting with specific antibodies directed against contact system zymogen forms demonstrated that FXII is activated by polyP, generates thrombin and fibrin via the intrinsic pathway of coagulation in vitro [80]. Do polyP trigger fibrin formation in vivo? In a model of lethal pulmonary embolism in genetically modified mice, FXII-deficient ($FXII^{-/-}$) and wild-type (WT, normal) mice treated with a protein-based FXII inhibitor (CSL829) survived injection of polyP. In contrast, the majority of WT and FXII-deficient mice that were reconstituted with human FXII (hFXII) before polyP treatment, died shortly after infusion of platelet polyP (Fig. 3).

Histological sections of lung tissue from polyP-treated mice show the majority of vessels were obstructed with clots in WT mice, virtually no thrombi were found in $FXII^{-/-}$ mice. These data show that polyP are procoagulant in an FXII-dependent manner in vivo. Phosphatase, which efficiently degrades polyP, interferes with procoagulant platelet activity. Activation of platelets largely reduced the clotting time of platelet-rich plasma as compared to recalcification times in the presence of unstimulated platelets and addition of phosphatase almost completely blocked the increased clotting activity conferred by stimulated platelets. Hermansky-Pudlak Syndrome (HPS) patients lack dense granules and can be viewed as a human model of platelet polyP deficiency. Fibrin formation in platelet-rich plasma is defective in HPS patients and these individuals suffer from increased bleeding. Consistently, stimulated platelets from HPS patients failed to shorten the clotting time in platelet-free plasma compared to normal platelets, presumably due to lack of polyP. Indeed, addition of exogenous platelet (or synthetic) polyP restored the defective clotting in these patients and shortened the time to clot formation to a "normal" time. These results demonstrate a role of polyP for fibrin production in patients and indicate that the concentration of polyP found in normal platelets is sufficient to trigger plasma coagulation, but that a reduction in polyP, such as in HPS platelets, impairs the procoagulant activity of activated platelets.

In contrast to heparin [61] and misfolded protein aggregates [81], polyP initiate FXII-dependent clotting and potently activate the kallikrein–kinin pathway generating BK. Bacterial polyP initiate lethal hypotonic reactions in a FXII-dependent manner [22] and platelet-derived polymers initiate skin edema via BK signaling. Overall, our findings identify inorganic polyP as a new class of platelet-derived procoagulant and proinflammatory mediator in vivo. PolyP activate the BK-forming kallikrein–kinin system and trigger fibrin formation on activated platelets in an FXII-dependent manner. The inorganic polymer is the long sought link of primary and secondary hemostasis [82]. Furthermore, polyP is a heretofore unrecognized target for a safe anticoagulation. Interrupting polyP-driven activation of the FXII-driven contact system efficiently and selectively interferes with thrombosis and edema formation in vivo and thus may present a new paradigm to fight thromboembolic and proinflammatory disease.

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