

Editorial Focus

A renaissance for the contact system in blood coagulation?

Yuval Blat¹, Dietmar Seiffert²

¹Department of Chemical Enzymology and ²Department of Thrombosis Biology, Bristol-Myers Squibb Company, Pennington, New Jersey, USA

A number of recent studies call for a reevaluation in our perception of the role of the contact activation system in blood coagulation. The antithrombotic phenotype of factor (F) XII-knockout mice and the identification of RNA and polyphosphates as physiological surfaces for contact activation provide new evidence that the contact system contributes to blood coagulation *in vivo*. The demonstration that feedback activation does not occur in plasma environment or the platelet surface, *in vitro*, leaves the contact system as the only known activator of FXI, currently. The complex prothrombotic, antithrombotic and profibrinolytic activities of the contact system may provide an explanation for the lack of bleeding in FXII, prekallikrein and high-molecular-weight kinogen (HK)-deficient individuals even in the absence of FXI feedback activation.

The contact activation system

It has long been recognized that the exposure of blood to artificial surfaces such as glass leads to rapid activation of the coagulation cascade and clot formation. The plasma proteins responsible for this phenomenon were termed the contact activation system (or the contact system, Fig. 1). Contact activation is initiated by the auto activation of FXII on a negatively charged surface. Activated FXII (FXIIa) activates plasma prekallikrein (PK) to plasma kallikrein, forming a positive feedback mechanism as plasma kallikrein activates additional FXII. Concurrently, FXIIa proteolytically activates FXI. Activated FXI (FXIa) is an efficient activator of FIX which in turn activates the common pathway of blood coagulation with resultant clot formation. In addition to the two serine proteases, FXIIa and plasma kallikrein, an additional auxiliary protein, HK, is essential for efficient activation of the contact system. FXI and PK circulate in binary complexes with HK. HK is not only required for their activation

but also serves as a substrate for plasma kallikrein (see [1–3] for recent detailed reviews of the contact activation system).

Is the contact activation system physiologically relevant for blood coagulation?

In spite of the robust effects of the contact activation system on blood coagulation *in vitro*, the relevance of this pathway to blood coagulation *in vivo* has been questioned. Individuals with deficiencies in the contact activation proteins, FXII, PK and HK, do not appear to suffer excessive bleeding (reviewed in [1, 4]). Furthermore, negatively charged artificial surfaces that efficiently activate the contact system *in vitro*, such as glass and dextran sulfate, may not have physiologically relevant counterparts. Indeed, an alternative mechanism for contact activation on the surface of endothelial cells was identified. Binding of HK/PK complex to the surface of endothelial cells via gC1qR, urokinase plasminogen activator receptor or cytokeratin 1 leads to PK activation by endothelial cells-associated prolylcarboxypeptidase (reviewed in [3]). The plasma kallikrein formed by this pathway catalyzes the hydrolysis of HK in a number of sites forming bradykinin (BK) as well as other cleavage products. FXII, although not required for initiation, may still participate in this or any other alternative pathway as FXII-knockout mice show reduced BK levels even in the absence of externally added contact activator (5).

Most noteworthy among HK proteolysis products is the peptide, BK, which mediates vasodilation by binding to its receptors. Plasma kallikrein also affects vasodilation through the renin-angiotensin system by activating prorenin to renin. Other intersection points between the renin-angiotensin system and the contact activation system have also been described (3). The role

Correspondence to:

Yuval Blat
Department of Chemical Enzymology
Bristol-Myers Squibb Company
311 Pennington-Rocky Hill Rd, Pennington, NJ 08534, USA
Tel.: +1 609 818 5158, Fax: +1 609 818 6935
E-mail: yuval.blat@bms.com

or

Dietmar Seiffert
Department of Thrombosis Biology
Bristol-Myers Squibb Company
311 Pennington-Rocky Hill Rd, Pennington, NJ 08534, USA
Tel.: +1 609 818 5148, Fax: +1 609 818 7877
E-mail: dietmar.seiffert@bms.com

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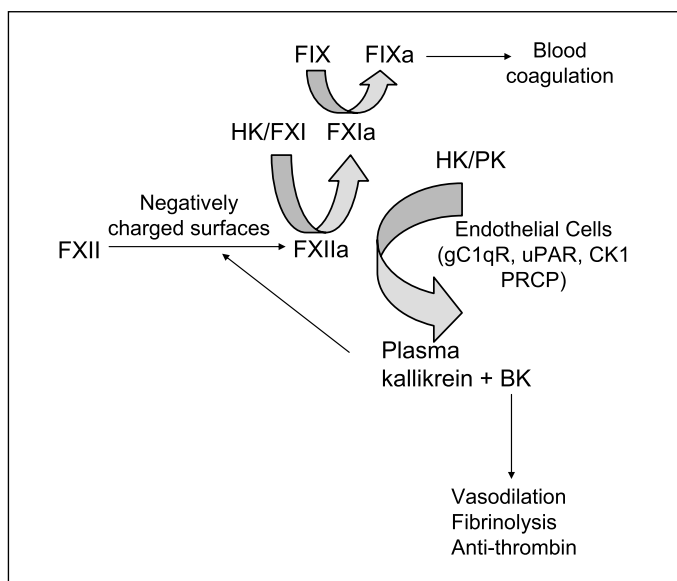


Figure 1: A schematic diagram of the activation of the contact system and its intersection with blood coagulation and vasodilation. gC1qR, globular C1q receptor; uPAR, urokinase plasminogen activator receptor; CK1, cytokreatin 1; PRCP, prolylcarboxypeptidase; BK, bradykinin; HK, high-molecular-weight kininogen; PK, prekallikrein; FIX, factor IX; FIXa, activated factor IX; FXI, factor XI; FXIa, activated factor XI; FXII, factor XII; FXIIa, activated factor XII.

of the contact activation system in several pathological conditions appears to support vasodilation rather than coagulation as the main physiological function of this system. For example, individuals with deficiency in C1 inhibitor, the major inactivator of the proteases of the contact activation system, suffer from angioedema but do not display any coagulopathy (1, 2). In sepsis, the contact system is known to be activated, and there is evidence that the contact system contributes to the haemodynamic pathology. However, the contact system is not required for the development of disseminated intravascular coagulation during sepsis (3).

Evidence for an independent function of FXI

Unlike deficiency in components of the contact activation system, FXI deficiency causes a mild bleeding disorder termed haemophilia C. This bleeding tendency is more pronounced in organs with strong fibrinolytic activity (for reviews see [6, 7]). The apparent difference in the clinical manifestation of FXI deficiency, compared to components of the contact activation system, initiated a search for alternative activators of FXI. Indeed, FXI was shown to be activated by thrombin, with the activation being strongly augmented by negatively charged polymers (8, 9). Other studies, however, argued against the physiological relevance of this mechanism, as FXI activation by thrombin was not observed in platelet-poor plasma (PPP) (10) and was inhibited by HK and fibrinogen (8, 11).

Several later studies tipped the scale again in favor of thrombin-mediated activation as the physiologically relevant mechanism of FXI activation. A number of groups demonstrated that FXI contributes to clot formation and inhibits fibrinolysis in PPP

clotting assays stimulated by low concentrations of thrombin or tissue factor (12–16). FXI-deficient plasma had longer clotting times in these assays. A number of publications, primarily by the Walsh lab, demonstrated that the activation of FXI by thrombin is dramatically stimulated by activated platelets (17–19). These findings, in addition to revealing a physiological template for FXI activation, provided an apparent explanation for the lack of FXI activation by thrombin in PPP (10). Interestingly though, the FXI dependence described in PPP clotting assays above did not require the presence of platelets. The process of FXI activation by thrombin was termed feedback activation (16) and was proposed to serve as a positive feedback mechanism to augment the coagulation cascade and inhibit fibrinolysis.

Recent evidence that the contact system has physiological functions in blood coagulation

While the belief that FXI function in blood coagulation is mainly mediated by feedback activation is the currently held view in the field, several recent studies are beginning to paint a different picture. A detailed analysis of FXII-knockout mice revealed that these mice, like the FXI-knockout mice, are protected from thrombosis in several different models without excessive bleeding (20–22). Furthermore, FXII knockout was found to confer protection against ischemic brain injury with the FXI-knockout mice displaying a similar phenotype (23). Results with FXI/FXII-double knockout mice have not been published yet and will be critical to determine whether FXII indeed functions via FXI activation in these models. In an attempt to explain the discrepancy between these findings and the divergent phenotypes of FXI- and FXII-deficient humans with respect to bleeding, it was suggested that contact activation can contribute to pathological thrombosis, whereas feedback activation only promotes haemostasis (24). A mechanism that could account for this distinction was not offered.

The physiological relevance of feedback activation was further questioned by our group (25). Using a sensitive ELISA assay we found no detectable FXI activation in PPP or PRP (platelet-rich plasma) even when high thrombin and tissue factor (TF) concentrations were added. Moreover, we used a functional plasma coagulation cascade activation assay to demonstrate that the FXI-dependence observed after stimulation with thrombin and TF is the result of contact system activation during blood draw and plasma isolation. The FXI-dependence of these plasma coagulation assays was eliminated when corn trypsin inhibitor, a specific FXIIa inhibitor, was included in the blood collection tubes. Indeed, the papers that reported the discovery of platelet-mediated feedback activation of FXI were retracted recently, as the stimulation of FXI activation by thrombin on the platelet surface could not be reproduced (26, 27).

Another step in the renaissance of the contact activation system as a physiologically significant contributor to blood coagulation was completed by the identification of RNA and polyphosphates as activators of the contact system (28, 29). Polyphosphates are abundant components of platelet dense granules and are released during platelet activation (30). The activation of blood coagulation by polyphosphates, *in vitro*, was shown to be

FXII-dependent (29). The work of Kannemeier et al. (28) presents a strong case for RNA as a physiologically important activator of the contact system. In addition to demonstrating the procoagulant activity of RNA during in-vitro clotting assays, Kannemeier et al. showed induction of contact activation and thrombosis *in vivo* by exogenously added RNA. Furthermore, the authors demonstrated that RNase treatment is antithrombotic even in the absence of exogenously added RNA. This finding suggests that RNA, released by necrotic cells, apoptotic cells and/or platelets, contributes to thrombus formation *in vivo*.

Why do FXI-deficient individuals bleed, while deficiency in other components of the contact system does not cause bleeding?

The FXI feedback activation theory presented a simple explanation for the lack of bleeding in individuals deficient in FXII, PK and HK. In this section, we will provide alternative explanations for the difference between individuals deficient for one of the contact system proteins and FXI-deficient individuals. In mice, unlike humans, the phenotypes of FXI- and FXII-deficient animals are quite similar. Both FXI- and FXII-knockout mice are protected from thrombosis but display minimal effects on tail bleeding times (20–22). This phenotype is much more consistent than the human phenotype with the contact system being the main activator of FXI during blood coagulation. Currently available experimental data certainly do not exclude species differences in the coagulation cascade. However such differences might be very subtle as human FXII was shown to complement mouse FXII in knockout mice (21, 23). Moreover, cows with congenital FXI deficiency do show an increased tendency for

bleeding like humans (31). Nevertheless, one might argue that knockout mice are a “cleaner” system for evaluating the role of the contact system in blood coagulation. They offer complete elimination of the gene in question and a much more uniform genetic background than humans. Moreover, it is possible that the small number and ethnical bias of individuals with FXII, PK and HK deficiencies might have precluded the identification of a mild bleeding disorder.

Alternatively, the apparent phenotypic difference between FXI-deficient individuals and individuals lacking components of the contact system may be real. In this case, it is important to take into consideration additional functions of the contact activation system. HK hydrolysis products were shown to mediate several profibrinolytic and thrombin inhibiting effects (1–3). BK stimulates tissue plasminogen activator release from endothelial cells (32), while its stable degradation product BK_{1–5} inhibits thrombin-induced platelet activation (33). Plasma kallikrein and FXIa can also contribute to fibrinolysis by direct activation of plasmin. However, direct plasmin activation by plasma kallikrein and FXIa is weak and can only be demonstrated following inactivation of the plasma serine protease inhibitors by acidification (2). Thus, in individuals deficient in components of the contact activation system, namely FXII, PK and HK, the anticoagulation effect of the loss of contact activation of FXI may be compensated by the elimination of the profibrinolytic and the antithrombotic activities of the contact system. In FXI deficient individuals, on the other hand, the procoagulant component of the contact activation system is eliminated but the profibrinolytic and antithrombin effects, which occur upstream and independently of FXI, are maintained with a net result of a bleeding disorder which is mostly manifested in organs with high fibrinolytic activity.

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