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# The elusive physiologic role of Factor XII

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**Physiologic hemostasis upon injury involves many plasma proteins in a well-regulated cascade of proteolytic reactions to form a clot. Deficiency of blood coagulation Factors VIII, IX, or XI is associated with hemophilia. Factor XII (FXII) autoactivates by contact with a variety of artificial or biologic negatively charged surfaces (contact activation), resulting in blood coagulation and activation of the inflammatory kallikrein-kinin and complement systems. However, surprisingly, individuals deficient in FXII rarely suffer from bleeding disorders. Most biologic surfaces that activate FXII become expressed in disease states. Investigators have long searched for physiologic activators of FXII and its role in vivo. In this issue of the *JCI*, Maas et al. show that misfolded protein aggregates produced during systemic amyloidosis allow for plasma FXIIa and prekallikrein activation and increased formation of kallikrein–C1 inhibitor complexes, without Factor XIa activation and coagulation (see the related article beginning on page 3208). This study describes a novel biologic surface for FXII activation and activity, which initiates inflammatory events independent of hemostasis.**

In the early 1950s, Oscar Ratnoff and Joan Colopy observed a patient, John Hageman, whose blood, upon routine preoperative screening, was found to have prolonged clotting times in glass test tubes, even though Hageman had no history or symptoms of a bleeding disorder (1). The observation that something was missing in his blood, and that this factor changed upon exposure to glass, ushered in the notion that blood clotting factors circulate as inactive precursors that can be activated. Ratnoff, in collaboration with Earl Davie, identified that, in the disorder that became known as Hageman trait, a plasma serine protease later called Factor XII (FXII) was missing. Absence of FXII prevents the activation of the blood coagulation zymogen

FXI that, when activated to become FXIa, leads to the formation of Factor IXa — a key intermediary in the intrinsic pathway of coagulation. These seminal studies contributed to the presentation of their waterfall cascade hypothesis for the blood coagulation system; a similar hypothesis was proposed that same year by Robert MacFarlane (2, 3). Ratnoff and his collaborators went on to show that FXII, which alters its physical properties during activation, induces vasodilatation and vascular permeability. These studies encapsulate the major known properties of FXII (Figure 1), a protein that autoactivates upon exposure to negatively charged surfaces to become the enzyme Factor XIIa (α-FXIIa), which then activates FXI, prekallikrein (PK), and C1 esterase (a subunit of the complement cascade). The consequence of FXI activation by α-FXIIa is the initiation of a series of proteolytic reactions resulting in thrombin generation, which precedes clot formation. α-FXIIa activation of PK forms plasma kallikrein that

can reciprocally activate more FXII and liberate bradykinin from high-molecular-weight kininogen (HK). Bradykinin is a mediator of vasodilatation and increased vascular permeability (4). α-FXIIa when cleaved by plasma kallikrein forms Factor βXIIa (β-FXIIa), which then activates the macromolecular complex of the first component of complement, resulting in classic complement system activation; plasma kallikrein also directly activates complement components C3 and C5 (5, 6). Thus, the activation of FXII results in coagulation and complement activation with bradykinin liberation (Figure 1).
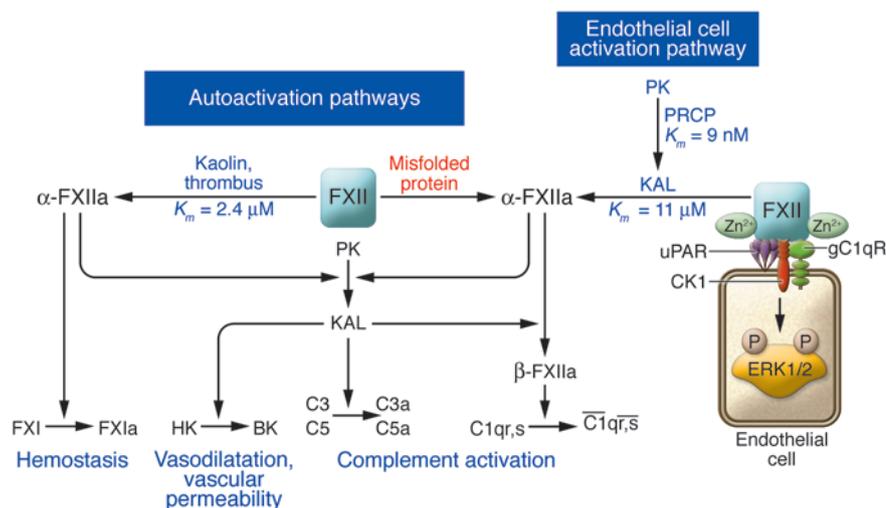
## Contact activation of Factor XII

Since FXII-deficient patients, along with PK- and HK-deficient patients, do not have a bleeding disorder, the relevance of the waterfall cascade hypothesis for blood coagulation hemostasis initiated by activated FXII has been questioned. Contact activation describes the unique property of FXII to undergo autoactivation and a change in shape when exposed to negatively charged artificial or biologic surfaces. Formation of FXIIa leads to PK activation in the presence of HK, hence the name *contact activation system*. However, the molecular basis for the formation of activated FXII remains unknown (7, 8). Over the last 4 decades, a growing list of "physiologic" negatively charged surfaces upon which FXII autoactivates have been recognized. In addition to nonphysiologic agents such as glass, polyethylene, and silicone rubber, FXII autoactivation occurs upon exposure to articular cartilage, skin, fatty acids, endotoxin, amyloid protein, and heparins, among others (9). It is in this context of identifying a biologic surface that sup-

**Figure 1**
Mechanisms for FXII activation. There are two pathways for FXII (Hageman factor) activation: autoactivation upon exposure to negatively charged surfaces and proteolytic activation on cell membranes. FXII autoactivates ($K_m = 2.4$ μM) on an artificial or biologic surface such as kaolin or a thrombus to activate FXII to α-FXIIa. α-FXIIa then activates FXI to FXIa to initiate hemostasis and activates PK to form plasma kallikrein (KAL). KAL cleaves HK to liberate bradykinin, which induces vasodilatation and vascular permeability. KAL also activates the complement system by directly activating complement components C3 and C5 and cleaving α-FXIIa to form β-FXIIa (a soluble light chain enzymatic form [Hageman factor fragment]), which then activates the macromolecular C1qr,s complex to enzymatically active C1r and C1s. The results of the study by Maas et al. in this issue of the *JCI* (10) suggest that a second FXII autoactivation mechanism occurs upon exposure of FXII to aggregates of misfolded proteins and that this activation results in PK activation without FXI activation — showing that the kallikrein-kinin system can be activated separately from the coagulation cascade by FXII. A second pathway for FXII activation occurs on endothelial cells. PK bound to HK on endothelial cells is activated to plasma KAL by the serine protease prolylcarboxypeptidase (PRCP) ($K_m = 9$ nM). KAL then activates FXII to α-FXIIa ($K_m = 11$ μM). FXII also binds to endothelial cells in the presence of zinc ions and when bound stimulates ERK1/2 phosphorylation. CK1, cytokeratin 1; gC1qR, gC1q receptor; uPAR, urokinase plasminogen activator receptor.

ports FXII autoactivation that the study by Maas et al. in this issue of the *JCI* should be viewed (10). Although many biologic substances allow for FXII autoactivation, their exposure to this zymogen usually does not occur constitutively in the intravascular compartment in non-disease states. An alternative hypothesis, which bypasses any role for FXII in physiologic blood coagulation, was proposed by Gailani and Broze, who showed that, in vitro, in the presence of dextran sulfate and HK, thrombin activates FXI (11). Although questions have arisen as to whether thrombin activation of FXI can actually occur in plasma due to its high fibrinogen concentration (thrombin proteolyzes fibrinogen and FXI with the same affinity, but fibrinogen is 2 orders of magnitude more abundant than FXI in plasma), the hemostasis community has adopted this latter mechanism to explain the hemostatic activity of FXI but not FXII (12–14). This revised model of the blood coagulation cascade has left researchers puzzling as to the physiological role of FXII and how it is activated in vivo. The present report by Maas et al. (10) provides insight into a novel activity for FXII, i.e., its ability to initiate an inflammatory response in the presence of aggregates of abnormal, misfolded proteins in vivo.

## Factor XII autoactivation initiated by misfolded or aggregated proteins

In the study in this issue by Maas et al. (10), the authors show that FXII autoactivation occurs in vitro in reaction to misfolded or amorphous protein aggregates of transthyretin (a homotetrameric protein in plasma and CSF), Bence-Jones protein (a monoclonal globulin protein found in blood or urine), glycated albumin, glycated hemoglobin, or the angiostatic drug endostatin (10). In individuals diagnosed with systemic amyloidosis, a condition characterized by the abnormal

deposition of misfolded amyloid proteins in organs and/or tissues, the authors observed significantly elevated levels of activated FXII compared with controls, as assessed by one commercially available assay (10). The authors go on to show that contact activation initiated by FXII in reaction to these misfolded proteins is associated with elevated levels of plasma kallikrein–C1 inhibitor complexes, but not FXIa–C1 inhibitor complexes, both in vitro and in patients with systemic amyloidosis (10). This latter finding suggests that PK activation (and consequent activation of the inflammatory kallikrein-kinin and complement systems) triggered by FXII autoactivation in reaction to misfolded or amorphous protein aggregates can proceed independently of the intrinsic coagulation pathway (Figure 1).

Do the results of the Maas et al. (10) study reveal a major new pathway for FXII autoactivation and provide insight regarding a physiologic activity for FXII? What is intriguing about the current investigation is that it demonstrates constitutive FXII contact activation in the disease state of systemic amyloidosis. The data also suggest that FXII autoactivation in reaction to misfolded protein initiates an inflammatory response in reaction to these abnormal protein deposits. Activation of the so-called contact system proteins has been recognized in other pathologic states, such as acute attacks of hereditary angioedema, sepsis, diabetic retinopathy, induced arterial thrombosis, and acute myocardial infarction. However, the authors suggest that in systemic amyloidosis, there is PK activation without FXI activation (10). This contact activation–mediated disease phenotype also has been recognized in acute attacks of hereditary angioedema, where PK activation and bradykinin formation occur without FXI activation.

There are some methodological questions pertaining to the current study that should be raised (10). Since the second-order rate constants of plasma kallikrein and FXIa inhibition by C1 inhibitor are similar, but the plasma concentration of PK is 18-fold higher than that of FXI, many more subjects would have had to be studied in the FXI group to conclusively show that FXI was not activated significantly in systemic amyloidosis. Further, the higher coefficient of variation of the controls in the FXIa–C1 inhibitor assay also may have contributed to the lack of differences seen in these patient samples.

The mechanism(s) by which PK, but not FXI, activation occurs in response to FXII autoactivation in reaction to misfolded proteins has not been elucidated in the current report (10). In future studies, it would be helpful to determine whether misfolded protein–triggered autoactivation of purified FXII results in reduced or absent activation of purified FXI compared with PK. Do misfolded proteins only bind HK and PK and not FXI? Finally, why do only aggregated proteins and not fibrillar proteins trigger FXII autoactivation?

FXII autoactivation in reaction to misfolded or amorphous protein aggregates in vivo, although a nonphysiologic event, may in fact constitute a type of detection or defense response to these structurally abnormal proteins. This pathway may constitute a means to regulate complement and inflammatory systems that involve coagulation protein S, thrombomodulin, and thrombin itself. Other biologic substances that have been reported to support FXII autoactivation and arise in disease states include RNA and sulfatides released after cell disruption and platelet polysomes present on a developing thrombus (15, 16). Unlike the present report (10), the previous two studies suggested that FXII autoactivation in reaction to a developing thrombus leads to FXI activation and thrombin formation (15, 16). The Maas et al. report suggests that certain biologic surfaces that trigger FXII autoactivation may lead to differential FXI or PK activation (10). Pathological contact activation in vivo is mostly associated with artificial substances interacting with the intravascular compartment. An extreme instance of this was reported recently: the infusion of unfractionated heparin adulterated with oversulfated chondroitin sulfate caused constitutional symptoms of nausea, dyspnea, and vomiting and 81 deaths from hypotension (17, 18). Oversulfated chondroitin sulfate, which in some heparin preparations was as high as 30% wt/wt, was associated with FXII autoactivation, plasma kallikrein formation, bradykinin liberation, and C3a and C5a formation, without evidence of FXI activation (18).

## Physiologic activation and activities of FXII and PK

An alternative to the contact activation hypothesis for FXII activation has been proposed. On cultured endothelial cells, the serine protease prolylcarboxypeptidase produces kinetically favorable PK activation ($K_m$ = 9 nM) versus PK activation by α-FXIIa on negatively charged surfaces ($K_m$ = 2.4 μM) (Figure 1) (19, 20). Formed plasma kallikrein then leads to favorable FXII activation ($K_m$ = 11 μM). FXII activation by plasma kallikrein on cells occurs at a faster rate than autoactivation (20). FXII is a multidomain protein with a surface cell-binding region that has an EGF-like growth factor region and a catalytic domain. It is structurally similar to EGF, single chain urokinase, and tissue plasminogen activator. In the intravascular compartment, FXII binds to endothelial cell urokinase plasminogen activator receptor, cytokeratin 1, and the complement receptor gC1qR (21) (Figure 1). FXII binding to endothelial membranes is highly regulated by the plasma concentration of HK and $Zn^{2+}$ (21). FXII, when bound to cells, stimulates ERK1/2 phosphorylation and [³H]thymidine uptake (22) (Figure 1).

Epidemiological studies in humans show that a polymorphism in FXII associated with lowered plasma FXII levels correlates with increased risk for myocardial infraction (23). These human clinical data do not correlate with the findings in FXII-deficient mice, which have shown reduced arterial thrombosis risk in several provocative mouse models (24). It has been proposed that the plasma kallikrein-kinin system influences thrombosis risk independent of hemostasis. Results from studies of both the kininogen- and bradykinin B2 receptor–knockout mice, in which the development of arterial thrombosis is delayed, support that assessment (25, 26).

In conclusion, the results of the present report by Maas et al. (10) indicate the existence of a pathway of FXII autoactivation upon its exposure to misfolded or aggregated proteins in the intravascular compartment or in tissues and that this pathway stimulates inflammatory systems without hemostasis activation. This pathway is not unlike that activated in acute attacks of hereditary angioedema, in which the absence of C1 inhibitor leads to increased contact system activation without hemostasis initiation. Alternatively, on a developing thrombus, FXII contributes to the size and strength of the occlusion. Additional studies are needed to better understand how there can be unique mechanisms of FXII autoactivation resulting in the formation of α-FXIIa that differentially activates its various substrates.

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# Platelet adhesion: a game of catch and release

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**The interaction of circulating platelets with the vessel wall involves a process of cell catch and release, regulating cell rolling, skipping, or firm adhesion and leading to thrombus formation in flowing blood. In this regard, the interaction of platelet glycoprotein Ibα (GPIbα) with its adhesive ligand, vWF, is activated by shear force and critical for platelet adhesion to the vessel wall. In this issue of the *JCI*, Yago and colleagues show how gain-of-function mutations in the GPIbα-binding vWF A1 domain disrupt intramolecular interactions within WT vWF A1 that regulate binding to GPIbα and flow-enhanced platelet rolling and adhesion (see the related article beginning on page 3195). Together, these studies reveal molecular mechanisms regulating GPIbα-vWF bond formation and platelet adhesion under shear stress.**

One of nature's mightiest forces is reptilian in origin. The gecko's foothold has a force of approximately 10 N/cm², allowing the gecko to more than support its own weight under the force of gravity (1). The biomechanics of this superb adaptation for reversible weight-bearing attachment to smooth surfaces involves hair-like structures (seta) on the footpad that end in multiple spatula-shaped projections capable of bearing approximately 20 μN per seta. Altering the 3D orientation of the seta with the surface (related to uncurling and peeling movements of the toe) can increase or decrease detachment forces (1).

No less remarkable in the mammalian world are structural adaptations at the molecular level controlling receptor-mediated cell adhesion, in particular cell attachment under hydrodynamic shear flow in the bloodstream (2–8). In the case of blood platelets, this involves the reversible interaction between platelet glycoprotein Ibα (GPIbα; the major ligand-binding subunit of the GPIb–IX-V complex) and vWF associated with an injured or diseased vessel wall. Fluid shear rates in flowing blood may be minimal at the center of the vessel, but become increasingly pronounced approaching the vessel wall as a result of drag (9). Shear stress on an adherent cell (force per unit area) depends on cell size, fluid viscosity, and proximity to the wall. At the cellular level, this has profound effects on initial contact, translocation, and firm adhesion of platelets under shear conditions. As shear rates increase from low physiological rates (less than 600 s⁻¹) to high physiological rates (up to about 1,500 s⁻¹) to pathological rates (up to about 10,000 s⁻¹, such as in a stenotic artery), contact adhesion of platelets is increasingly dependent on GPIbα/vWF, and elongated tether-like structures may form parallel to the direction of flow (10, 11).

To date, little is understood about the mechanical properties of individual GPIbα/vWF molecular bonds, or how congenital point mutations affecting GPIbα-vWF binding affinity determine the physical interaction. In their study in this issue of the *JCI*, Yago et al. use atomic force microscopy (AFM) and molecular dynamics simulations to show how orientation of the GPIb-binding A1 domain of vWF with GPIbα, involving alignment of specific amino acid residues, may regulate catch/slip bonding and release of GPIbα as shear force increases (8).

## vWF

vWF is a multifunctional adhesive glycoprotein stored in platelet α-granules and Weibel-Palade bodies of endothelial cells. In its mature form, it consists of disulfide-linked, approximately 275-kDa subunits forming multimers of at least 20,000 kDa (Figure 1). Each subunit is composed of conserved domains: D′-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2 (12). The A1 domain encompassing the disulfide bond at C1272–C1458 (C509–C695 in the mature sequence) contains the binding site for GPIbα, heparin, and other binding partners. The vWF A3 domain binds collagen, while C1 contains an Arg-Gly-Asp (RGD) sequence that binds the platelet integrin $\alpha_{IIb}\beta_3$. The GPIb-binding site on vWF A1 is cryptic under static conditions, but becomes competent to bind receptor when matrix associated, exposed to shear force, or activated by nonphysiological modulators such as the bacterial glycopeptide ristocetin or the snake toxin botrocetin (12, 13). Ultralarge vWF multimers expressed on activated endothelial cells