

The factor XIIa blocking antibody 3F7: a safe anticoagulant with anti-inflammatory activities

Marie Worm¹, Elodie C. Köhler¹, Rachita Panda¹, Andy Long¹, Lynn M. Butler^{1,2}, Evi X. Stavrou^{3,4}, Katrin F. Nickel^{1,2}, Tobias A. Fuchs^{1,2}, Thomas Renné^{1,2}

¹Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ²Division of Clinical Chemistry, Department of Molecular Medicine and Surgery and Center of Molecular Medicine, Karolinska Institutet and University Hospital, Stockholm, Sweden; ³Division of Hematology and Oncology, Department of Medicine, Case Western Reserve University, Cleveland, Ohio, USA; ⁴Department of Medicine, Louis Stokes Veterans Administration Hospital, Cleveland, Ohio, USA

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Correspondence to: Thomas Renné, MD, PhD. Clinical Chemistry, Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital Solna (L1:00), SE-171 76 Stockholm, Sweden; Institute of Clinical Chemistry and Laboratory Medicine (O26), University Medical Center Hamburg-Eppendorf, Martinistrasse 51, D-20246 Hamburg, Germany. Email: thomas@renne.net.

Abstract: The plasma protein factor XII (FXII) is the initiating protease of the procoagulant and proinflammatory contact system. FXII activates both the bradykinin (BK) producing kallikrein-kinin system and the intrinsic pathway of coagulation. Contact with negatively charged surfaces induces auto-activation of zymogen FXII that results in activated FXII (FXIIa). Various *in vivo* activators of FXII have been identified including heparin, misfolded protein aggregates, nucleic acids and polyphosphate. Murine models have established a central role of FXII in arterial and venous thromboembolic diseases. Despite the central function of FXII in pathologic thrombosis, its deficiency does not impair hemostasis in animals or humans. The selective role of FXIIa in thrombosis, but not hemostasis, offers an exciting novel strategy for safe anticoagulation based on interference with FXIIa. We have generated the recombinant fully human FXIIa-blocking antibody 3F7, which abolished FXIIa enzymatic activity and prevented thrombosis in a cardiopulmonary bypass system in large animals, in the absence of increased therapy-associated bleeding. Furthermore, 3F7 also interfered with BK-driven edema in the severe swelling disorder hereditary angioedema (HAE) type III. Taken together, targeting FXIIa with 3F7 appears to be a promising approach to treat edema disorders and thrombosis.

Keywords: Cardiovascular; coagulation; factor XII (FXII); thrombosis; anticoagulation; angioedema; hereditary angioedema (HAE) type III

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The contact system

The contact system is a cascade of five plasma proteins with procoagulant and proinflammatory activities. It consists of the serine proteases factor XII (FXII, Hageman factor), factor XI (FXI), plasma kallikrein (PK), the non-enzymatic cofactor high molecular weight kininogen (HK) and C1 esterase inhibitor (C1INH). Activated FXII (FXIIa) initiates the contact system that drives the intrinsic pathway of

coagulation and the inflammatory kallikrein-kinin system leading to fibrin and bradykinin (BK) formation, respectively (1,2). FXII zymogen is primarily released by hepatocytes and circulates in plasma as a single chain protein (3). FXII binding to negatively charged surfaces induces a conformational change. Surface bound FXII activates itself by limited proteolysis of the single peptide-bond Arg₃₅₃-Val₃₅₄ (auto-activation), leading to FXIIa, a two chain serine protease. A

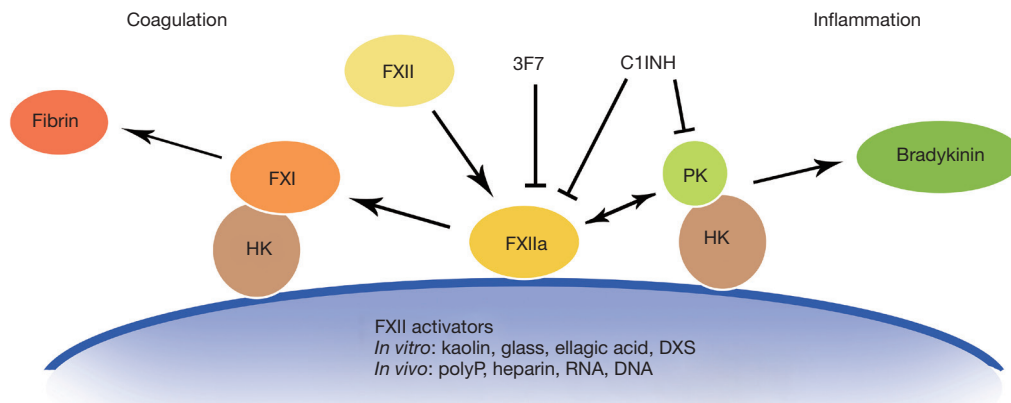


Figure 1 Contact with negatively charged surfaces activates FXII and initiates procoagulant and proinflammatory reactions. Activated FXII starts the fibrin producing intrinsic pathway of coagulation by cleavage of FXI and the inflammatory kallikrein-kinin system by PK mediated cleavage of HK. C1INH interferes both with FXIIa and PK while 3F7 specifically blocks FXIIa only. DXS, dextran sulphate; FXII, factor XII; PK, plasma kallikrein; C1INH, C1 esterase inhibitor.

single disulfide bond in each FXIIa molecule connects the heavy chain to the light chain. FXIIa cleaves its substrate FXI to generate activated FXI (FXIa) and plasma prekallikrein to PK. PK reciprocally activates FXII, leading to amplification of the process (4). Two further cleavage steps commencing at residues 334 and 343 release the light chain from the heavy chain that remains surface bound (5). The contact system proteins locally assemble on surfaces of various cardiovascular cells. While FXII binds directly to surfaces, HK is required to bind FXI and PK (6,7). C1INH is the principle endogenous inhibitor of FXIIa and PK and regulates the enzymatic activity of the contact system proteases (8) (Figure 1).

FXII in coagulation

Fibrin production by the classic coagulation cascade can be initiated by two distinct mechanisms involving vessel wall (extrinsic) or blood borne (intrinsic) factors. Both pathways lead to the formation of thrombin, a protease that converts fibrinogen to insoluble fibrin. The extrinsic pathway is initiated when tissue factor (TF) is exposed at sites of vascular injury and binds to circulating coagulation factor VII (FVII) (9). In contrast, the intrinsic pathway is initiated by contact mediated activation of FXII and subsequent cleavage of FXI (10).

A commonly used diagnostic coagulation test, the activated partial thromboplastin time (aPTT), is based on FXII-mediated contact activation. It is used to monitor coagulation in patients treated with heparin, and it is prolonged in the presence of lupus anticoagulant and in inherited or acquired deficiencies in contact system

proteins. Despite its high relevance *in vitro*, FXII was considered to have no function for coagulation *in vivo*. In contrast to all other coagulation proteases, deficiency in FXII in humans does not lead to any bleeding disorder, despite a marked prolongation of the aPTT (11,12). Studies on FXII deficient (FXII^{-/-}) mice revealed an essential role of FXII in thrombosis. FXII^{-/-} mice were largely protected from vessel occlusive thrombus formation in venous and arterial vascular beds in response to chemical or mechanical endothelial injury (13-15). Like their human counterparts, FXII^{-/-} mice have a normal hemostatic capacity but a prolonged aPTT (16). Substitution of FXII^{-/-} mice with human FXII normalized the prolonged aPTT and restored defective thrombus formation (13), indicating that FXII operates similarly in mice and humans. In addition to triggering fibrin production, direct binding of FXIIa to fibrin(ogen) modulates the fibrin clot structure leading to increased fiber density and reduced fibrin meshwork pore size (17).

FXII in hereditary angioedema (HAE)

The rare inherited swelling disorder HAE is caused by excessive BK signalling (8). HAE is characterized by acute swelling attacks involving the skin, the oropharyngeal, laryngeal or gastrointestinal mucosa. Swelling events can be life-threatening due to development of laryngeal edema and airway compromise (18). The classic HAE types 1 and 2 (HAE I and HAE II) are caused by deficiencies of C1INH, the major plasma inhibitor of FXIIa and PK. HAE

I and HAE II result due to deficiency in or functional loss of C1INH and hence, these HAE types can be treated by infusion of functional C1INH (8).

A third variant of HAE exists and despite the fact that patients suffer from similar symptoms as HAE types I and II, normal plasma levels of a fully functional C1INH are found in HAE type III patients (19). HAE III is associated with a single missense mutation in the *F12* gene leading to an amino acid exchange, Thr309Lys (20). It was subsequently shown that Thr309 could also be mutated to an Arginine (21). The underlying mechanism of HAE III has been enigmatic for over a decade; recently defective glycosylation in the two mutated FXII forms was identified. Contact activation is largely increased in mutated FXII variants, leading to excess BK formation in patient plasma. Both activated mast cell and contact-triggered edema was largely increased in FXII^{-/-} mice infused with recombinant FXII_Thr309Lys or FXII_Thr309Arg and a novel transgenic mouse with inducible expression of human FXII_Thr309Lys. Together, these data show that loss of glycosylation leads to an increased contact-induced activation of Thr309-mutated FXII that triggers excessive BK formation. Of note, aPTT and thrombus formation were normal in HAE III patient plasma and HAE III mouse models (22).

FXII activators

Binding to negatively charged surfaces activates zymogen FXII. Various *in vitro* activators of FXII have been identified such as the white clay material kaolin that is used in diagnostic assays (4). Other non-physiologic activators include glass or ellagic acid and synthetic phospholipid micelles decorated with bivalent cations (23). Moreover, exposure of blood to polymer surfaces exposed in medical devices can lead to FXII activation. Clinical procedures such as hemodialysis and extracorporeal circulation that expose blood to large non-biologic surfaces trigger FXIIa generation and thus, are associated with a high thrombotic risk requiring anticoagulant treatment (24). The synthetic polysaccharide high molecular weight dextran sulphate (DXS) is commonly used in experimental settings to activate FXII. It exclusively activates the kallikrein-kinin system without interfering with coagulation (2). Similarly to DXS, the mast cell-derived polysaccharide heparin has the ability to trigger contact system activation. IgE/antigen activated mast cells release heparin in allergic disease animal models leading to BK formation without causing increased coagulation (25). Consistently, activated mast cell-driven BK formation via the

contact system critically contributes to anaphylaxis and possibly other allergic diseases (26). Insoluble misfolded aggregated proteins selectively activate the kallikrein-kinin system without triggering coagulation (27) and BK formed by this mechanism contributes to inflammatory reactions seen in Alzheimer disease (28). Extracellular RNA and DNA have been identified as naturally occurring FXII activators that trigger coagulation and cleavage of the phosphoester bonds in the polymer backbone; use of RNase provided thromboprotection in a murine thrombosis model (29). Activated neutrophils can undergo a cell death program that leads to the formation of neutrophil extracellular traps (NETs) consisting of nuclear DNA with histones and microbicidal proteins. This process is called NETosis and constitutes a host defence mechanism against microbial pathogens which become trapped in the NETs (30). The ability of large DNA clusters to activate FXII (31) has been implicated in both macrovascular (32) and microvascular thrombosis (33). Activated platelets release the inorganic polymer polyphosphate that is stored in platelet dense granules (34). Synthetic platelet size polyphosphate triggers coagulation in a FXII-dependent manner *in vitro* (35). Human plasma experiments, thrombosis and edema models in mice revealed that polyphosphate FXIIa *in vivo* and interference with the polyphosphate-FXII pathway interfered with thrombosis and vascular leakage (36). Supporting the critical role of polyphosphate in FXII activation, inherited deficiency in the polymer delays clotting in platelet rich plasma and thrombosis in Hermansky-Pudlak syndrome patients (37) and inositol hexakisphosphate kinase 1 deficient mice (38), respectively. The polyphosphate-FXII pathway operates independently of TF-driven (extrinsic) coagulation (39). Recent studies using genetically modified mice and patient plasma have shown a critical role of the polyphosphate-FXII pathway in prostate cancer associated thrombosis. Polyphosphates on cancer cells and derived microparticles potently activate FXII and drive venous thrombosis *in vivo* (40). Consistently, platelet derived microparticle-driven clotting is critically dependent on FXII suggesting that microparticles from platelets also expose polyphosphate on their surface (41).

Targeting FXII

The fact that FXII is essential for thrombosis but dispensable for hemostasis offers the first safe strategy for anticoagulation. We produced the fully human recombinant antibody 3F7 that specifically binds to the catalytic domain of FXIIa with high affinity and completely inhibits its

protease activity. Antibody 3F7 blocks FXIIa-mediated clotting in human plasma and thrombosis in mouse models. We adapted an extracorporeal membrane oxygenation (ECMO) system, used for infant therapy, and applied it to a rabbit model. 3F7 provided thromboprotection as efficiently as heparin, however, in sharp contrast to heparin, 3F7 did not increase bleeding (42). Furthermore, targeting FXIIa with 3F7 interfered with edema in a humanized HAE III mouse model and BK-formation in HAEIII patient plasma (22). Currently, treatments for acute edema attacks include infusion of C1INH (43), inhibition of kallikrein with ecallantide (DX-88) (44) and targeting the BK receptor B2R (icatibant) (45). Inhibition of FXIIa with 3F7 could represent a novel therapeutic strategy both in the prophylaxis and acute edema attack stages of HAE (22). Clinical applications of 3F7 require further investigation and will present new possibilities for a novel safe drug to potently interfere with thrombosis and inflammation.

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Footnote

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References

- Renné T. The procoagulant and proinflammatory plasma contact system. *Semin Immunopathol* 2012;34:31-41.
- Renné T, Schmaier AH, Nickel KF, et al. In vivo roles of factor XII. *Blood* 2012;120:4296-303.
- Stavrou E, Schmaier AH. Factor XII: what does it contribute to our understanding of the physiology and pathophysiology of hemostasis & thrombosis. *Thromb Res* 2010;125:210-5.
- Maas C, Renné T. Regulatory mechanisms of the plasma contact system. *Thromb Res* 2012;129 Suppl 2:S73-6.
- Kenne E, Renné T. Factor XII: a drug target for safe interference with thrombosis and inflammation. *Drug Discov Today* 2014;19:1459-64.
- Renné T, Gailani D, Meijers JC, et al. Characterization of the H-kininogen-binding site on factor XI: a comparison of factor XI and plasma prekallikrein. *J Biol Chem* 2002;277:4892-9.
- Renné T, Dedio J, Meijers JC, et al. Mapping of the discontinuous H-kininogen binding site of plasma prekallikrein. Evidence for a critical role of apple domain-2. *J Biol Chem* 1999;274:25777-84.
- Björkqvist J, Sala-Cunill A, Renné T. Hereditary angioedema: a bradykinin-mediated swelling disorder. *Thromb Haemost* 2013;109:368-74.
- Mackman N. Tissue-specific hemostasis: role of tissue factor. *J Thromb Haemost* 2008;6:303-5.
- Renné T, Oschatz C, Seifert S, et al. Factor XI deficiency in animal models. *J Thromb Haemost* 2009;7 Suppl 1:79-83.
- Lämmle B, Wuillemin WA, Huber I, et al. Thromboembolism and bleeding tendency in congenital factor XII deficiency--a study on 74 subjects from 14 Swiss families. *Thromb Haemost* 1991;65:117-21.
- Müller F, Gailani D, Renné T. Factor XI and XII as antithrombotic targets. *Curr Opin Hematol* 2011;18:349-55.
- Renné T, Pozgajová M, Grüner S, et al. Defective thrombus formation in mice lacking coagulation factor XII. *J Exp Med* 2005;202:271-81.
- Stavrou EX, Fang C, Merkulova A, et al. Reduced thrombosis in Klkb1^{-/-} mice is mediated by increased Mas receptor, prostacyclin, Sirt1, and KLF4 and decreased tissue factor. *Blood* 2015;125:710-9.
- Kleinschnitz C, Stoll G, Bendszus M, et al. Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. *J Exp Med* 2006;203:513-8.
- Pauer HU, Renné T, Hemmerlein B, et al. Targeted deletion of murine coagulation factor XII gene--a model for contact phase activation in vivo. *Thromb Haemost* 2004;92:503-8.
- Konings J, Govers-Riemslog JW, Philippou H, et al. Factor XIIa regulates the structure of the fibrin clot independently of thrombin generation through direct interaction with fibrin. *Blood* 2011;118:3942-51.
- Tse K, Zuraw BL. Recognizing and managing hereditary angioedema. *Cleve Clin J Med* 2013;80:297-308.
- Bork K, Barnstedt SE, Koch P, et al. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet* 2000;356:213-7.
- Cichon S, Martin L, Hennies HC, et al. Increased activity of

- coagulation factor XII (Hageman factor) causes hereditary angioedema type III. *Am J Hum Genet* 2006;79:1098-104.
21. Dewald G, Bork K. Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. *Biochem Biophys Res Commun* 2006;343:1286-9.
 22. Björkqvist J, de Maat S, Lewandrowski U, et al. Defective glycosylation of coagulation factor XII underlies hereditary angioedema type III. *J Clin Invest* 2015;125:3132-46.
 23. Mutch NJ, Waters EK, Morrissey JH. Immobilized transition metal ions stimulate contact activation and drive factor XII-mediated coagulation. *J Thromb Haemost* 2012;10:2108-15.
 24. Svensson M, Friberger P, Lundström O, et al. Activation of FXII during haemodialysis. *Scand J Clin Lab Invest* 1996;56:649-52.
 25. Oschatz C, Maas C, Lecher B, et al. Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo. *Immunity* 2011;34:258-68.
 26. Sala-Cunill A, Björkqvist J, Senter R, et al. Plasma contact system activation drives anaphylaxis in severe mast cell-mediated allergic reactions. *J Allergy Clin Immunol* 2015;135:1031-43.e6.
 27. Maas C, Govers-Riemslog JW, Bouma B, et al. Misfolded proteins activate factor XII in humans, leading to kallikrein formation without initiating coagulation. *J Clin Invest* 2008;118:3208-18.
 28. Zamolodchikov D, Chen ZL, Conti BA, et al. Activation of the factor XII-driven contact system in Alzheimer's disease patient and mouse model plasma. *Proc Natl Acad Sci U S A* 2015;112:4068-73.
 29. Kannemeier C, Shibamiya A, Nakazawa F, et al. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. *Proc Natl Acad Sci U S A* 2007;104:6388-93.
 30. Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol* 2007;176:231-41.
 31. Oehmcke S, Mörgelin M, Herwald H. Activation of the human contact system on neutrophil extracellular traps. *J Innate Immun* 2009;1:225-30.
 32. Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A* 2010;107:15880-5.
 33. Jiménez-Alcázar M, Napirei M, Panda R, et al. Impaired DNaseI-mediated degradation of neutrophil extracellular traps is associated with acute thrombotic microangiopathies. *J Thromb Haemost* 2015;13:732-42.
 34. Ruiz FA, Lea CR, Oldfield E, et al. Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes. *J Biol Chem* 2004;279:44250-7.
 35. Smith SA, Mutch NJ, Baskar D, et al. Polyphosphate modulates blood coagulation and fibrinolysis. *Proc Natl Acad Sci U S A* 2006;103:903-8.
 36. Müller F, Mutch NJ, Schenk WA, et al. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. *Cell* 2009;139:1143-56.
 37. Müller F, Renné T. Platelet polyphosphates: the nexus of primary and secondary hemostasis. *Scand J Clin Lab Invest* 2011;71:82-6.
 38. Ghosh S, Shukla D, Suman K, et al. Inositol hexakisphosphate kinase 1 maintains hemostasis in mice by regulating platelet polyphosphate levels. *Blood* 2013;122:1478-86.
 39. Nickel KF, Spronk HM, Mutch NJ, et al. Time-dependent degradation and tissue factor addition mask the ability of platelet polyphosphates in activating factor XII-mediated coagulation. *Blood* 2013;122:3847-9.
 40. Nickel KF, Ronquist G, Langer F, et al. The polyphosphate-factor XII pathway drives coagulation in prostate cancer-associated thrombosis. *Blood* 2015;126:1379-89.
 41. Kiliç E, Van Oerle R, Borissoff JI, et al. Factor XII activation is essential to sustain the procoagulant effects of particulate matter. *J Thromb Haemost* 2011;9:1359-67.
 42. Larsson M, Rayzman V, Nolte MW, et al. A factor XIIa inhibitory antibody provides thromboprotection in extracorporeal circulation without increasing bleeding risk. *Sci Transl Med.* 2014;6:222ra17.
 43. Zuraw BL, Busse PJ, White M, et al. Nanofiltered C1 inhibitor concentrate for treatment of hereditary angioedema. *N Engl J Med* 2010;363:513-22.
 44. Cicardi M, Levy RJ, McNeil DL, et al. Ecallantide for the treatment of acute attacks in hereditary angioedema. *N Engl J Med* 2010;363:523-31.
 45. Cicardi M, Banerji A, Bracho F, et al. Icatibant, a new bradykinin-receptor antagonist, in hereditary angioedema. *N Engl J Med* 2010;363:532-41.

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