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The Intrinsic Pathway of Coagulation as a Target for Antithrombotic Therapy

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Abstract

Plasma coagulation in the activated partial thromboplastin time assay is initiated by sequential activation of coagulation factors XII, XI and IX – the classical intrinsic pathway of coagulation. It is well recognized that this series of proteolytic reactions is not an accurate model for hemostasis *in vivo*, as factor XII deficiency does not cause abnormal bleeding, and fXI deficiency causes a relatively mild propensity to bleed excessively with injury. Despite their limited roles in hemostasis, there is mounting evidence that fXI and fXII contribute to thrombosis, and that inhibiting them can produce an antithrombotic effect with a relatively small effect on hemostasis. In this chapter the contributions of components of the intrinsic pathway to thrombosis in animal models and humans are discussed, and results of early clinical trials of drugs targeting factors IX, XI and XII are presented.

Keywords

Intrinsic Pathway; Contact Activation; Thrombosis; Factor XI; Factor XII

Introduction

The protease thrombin makes essential contributions to hemostasis through its capacity to catalyze conversion of fibrinogen to fibrin, to stimulate platelet and vascular endothelial cells, and to activate plasma coagulation factors.[1] Thrombin also plays a central role in thrombosis, and several approaches have been developed to manipulate this enzyme to achieve an antithrombotic effect. The activity of thrombin or factor Xa (fXa, the enzyme

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responsible for converting prothrombin to thrombin) can be inhibited directly with drugs targeting the enzyme active sites (argatroban, dabigatran, and bivalirudin for thrombin; rivaroxaban, epixaban, and edoxaban for fXa),[2] or indirectly with heparin-related compounds (heparin, low molecular weight, fondaparinux) that enhance the activity of the plasma inhibitor antithrombin.[3] Alternatively, synthesis of prothrombin and factor X (fX), the precursors of thrombin and fXa, can be reduced with vitamin K antagonists such as warfarin.[4] Use of these effective antithrombotic strategies comes with a well recognized cost. Thrombin and fXa serve vital roles in hemostasis, and therapies directed at them will increase bleeding. Use of heparin is associated with major bleeding rates of up to 3%, and with warfarin 2-13%.[3-5] Newer oral thrombin and fXa inhibitors appear to cause less bleeding than the older drugs, and are easier to use.[2,6] However, because of their mechanisms of action, there are limits on the types of patients who are eligible to receive them, the clinical settings in which they can be used, and the intensity of anticoagulation that can be applied.

The strategy of targeting thrombin and/or fXa to achieve an antithrombotic effect is based on the intuitive notion that formation of an intravascular thrombus is largely the result of dysregulation of processes normally involved in hemostasis. This premise is currently being reconsidered. There is substantial interest in developing and testing novel therapies that target the proteases of the plasma intrinsic pathway of coagulation (factor IX [fIX], factor XI [fXI], factor XII [fXII], and prekallikrein [PK]) for treating or preventing thromboembolic disorders.[7-9] The physiologic importance of the intrinsic pathway has been questioned since the original descriptions of the cascade-waterfall model of coagulation[10,11] because, while some components of the pathway are clearly required for hemostasis, others are not.[12] This chapter reviews pre-clinical and clinical data supporting the hypothesis that components of the intrinsic pathway, and perhaps the intrinsic pathway itself, contributes to thrombosis; and that a useful antithrombotic effect can be achieved by targeting plasma factors that serve relatively minor roles in hemostasis. To understand how the intrinsic pathway might contribute to thrombosis, we first need to review how our understanding of this pathway has evolved over the past fifty years.

The Intrinsic Pathway in Models of Blood Coagulation

The Cascade-Waterfall Model of Thrombin Generation

The cascade-waterfall hypotheses of “intrinsic” coagulation was first proposed in two landmark papers in 1964 by R. Gwynn Macfarlane, and by Earl David and Oscar Ratnoff.[10-12] In subsequent models based on this scheme, the process of thrombin generation is the result of amplification of a procoagulant signal initiated by conversion of fXII to factor XIIa (fXIIa), followed sequentially by activation of the enzyme precursors fXI, fIX, fX and prothrombin (**Figure 1A**). At the time this model was proposed, it was recognized that much of the cascade could be bypassed through a process involving factor VIIa (fVIIa) (**Figure 1A**),[10,11] but the relative importance of this to intrinsic coagulation was not clear. The scheme in **Figure 1A** depicts the major enzyme reactions that contribute to plasma clotting in the activated partial thromboplastin time (aPTT) and prothrombin time (PT) assays used in clinical practice. There are two triggering mechanisms that converge at

the level of fX activation. Activation through the intrinsic pathway (**Figure 1A**, yellow arrows) is assessed by the aPTT assay, and is initiated by a process involving fXII called contact activation (discussed below). In the PT assay, coagulation is triggered through the extrinsic pathway by adding tissue extracts to the plasma that contain tissue factor (TF),[13] a cofactor for fVIIa.

The sequential step-like nature of the coagulation cascade implies that complete deficiency of any component would break the reaction chain and cause bleeding, but this is not what is observed in clinical practice.[13] Absence of fIX or its cofactor factor VIII (fVIII) causes the severe bleeding disorder hemophilia, implying an important role for the intrinsic pathway in hemostasis.[14] However, complete fXII deficiency, despite causing a marked prolongation of the aPTT, does not cause abnormal bleeding;[7,12] while fXI deficiency causes a relatively mild hemorrhagic disorder that involves tissues distinct from those commonly affected in fIX or fVIII deficiency.[12,15,16] These observations indicate that the classic intrinsic pathway does not accurately describe the manner in which its components contribute to hemostasis, and have lead to revisions in the models that are more in line with clinical phenotypes.

Tissue Factor-Initiated Thrombin Generation – The Role of Factor XI

It is the current consensus that thrombin generation at a site of vascular injury is initiated primarily by fVIIa in complex with TF (**Figure 1B**), an integral membrane protein expressed on cells underlying blood vessel endothelium.[17,18] FVIIa/TF initiates coagulation by activating fX, as in the PT assay (**Figure 1A**), and also activates fIX, which sustains fXa and thrombin production. The protease precursors (prothrombin, fVII, fIX and fX) and cofactors (TF, fVa and fVIIIa) shown within the gray area in **Figure 1B** form the core mechanism for thrombin generation in almost all vertebrate organisms.[19] Total deficiency of any one of these proteins results in a severe bleeding disorder or is not compatible with life.[20] This core mechanism is the target of all currently approved anticoagulants. Thus, it is to be expected that use of these compounds will increase bleeding risk.

In addition to the core proteins mammals have fXI, the precursor of the protease factor XIa (fXIa) (**Figure 1B**).[21,22] Severe fXI deficiency may cause excessive bleeding with trauma, particularly if tissues with robust fibrinolytic activity such as the oropharynx or urinary tract are involved.[12,15,16] But unlike fIX deficiency, spontaneous bleeding is not common in fXI deficiency. Furthermore, bleeding is highly variable, and some fXI deficient individuals are asymptomatic despite marked abnormalities in the aPTT. While fXI is activated by fXIIa in the cascade-waterfall model (**Figure 1A**), the absence of abnormal bleeding in fXII deficient individuals indicates other mechanisms for fXI activation must exist. For example, thrombin generated early in coagulation can activate fXI (**Figure 1B**, green arrow).[23-25]

In the scheme shown in **Figure 1B**, fXIa sustains thrombin generation by activating fIX, rather than contributing to initiation of thrombin formation, as in the cascade-waterfall model and the aPTT assay. A key feature of the newer model is that there are two mechanisms for fIX activation, explaining the differences in bleeding phenotypes associated with fIX and fXI deficiencies. In the absence of fXI, fIX still contributes to thrombin production because it can be activated by factor VIIa/TF. Indeed, clinical observations would suggest that factor VIIa/TF is probably the more important mechanism for fIX activation.

Along similar lines, the different phenotypes associated with fXI and fXII deficiency are explained by the presence of more than one mechanism for fXI activation. Current models of thrombin generation often do not include a role for fXII, based largely on the observation that fXII deficiency is not associated with a bleeding diathesis. However, as we will see, it does not necessarily follow that fXIIa does not contribute to thrombin generation under any circumstances.

The Kallikrein-Kinin System and Contact Activation

The plasma protease precursors fXII and PK and the cofactor high molecular weigh kininogen (HK) form the plasma kallikrein-kinin system.[26,27] FXII was first identified as a plasma constituent missing in a patient with a very long aPTT, but without abnormal bleeding. Subsequent work identified PK and HK as necessary for normal fXII activation in the aPTT. When plasma is exposed to artificial surfaces or anionic substances, fXII and PK undergo reciprocal conversion to fXIIa and α-kallikrein by a process called contact activation (**Figure 1C**).[12,26,27] HK serves as a cofactor during this process. In the aPTT assay, a purified earth such as silica or celite is used to induce contact activation, with the resulting fXIIa promoting thrombin generation by activating fXI. The absence of bleeding symptoms in individuals lacking fXII, PK or HK demonstrates that these proteins are not required for hemostasis,[12] either because they do not normally contribute to thrombin generation, or because other mechanisms such as thrombin-mediated feedback activation of fXI (**Figure 1B**) compensate for their absence. The kallikrein-kinin system is thought to contribute to a number of homeostatic and host-defense mechanisms including inflammation and the innate immune response to microorganisms.[26-29] It is also clear that fXIIa and fXIa, despite their limited roles in hemostasis, are required for thrombosis in experimental animal models. If these proteins prove to be important contributors to thrombosis in humans, inhibiting them may produce an antithrombotic effect without significantly affecting hemostasis. The following section reviews what is known about the intrinsic pathway proteins in thrombosis in animal models and in human populations.

The Intrinsic Pathway and Thrombosis

Animal Models of Thrombosis

Mice lacking individual components of the intrinsic pathway, including the kallikrein-kinin system, have been tested for resistance to thrombus formation. FIX deficient mice have a significant bleeding disorder (the murine equivalent of hemophilia B),[30] and are resistant to thrombus formation induced by injuring blood vessels with concentrated ferric chloride ($FeCl_3$).[30,31] In contrast, mice lacking fXI or fXII do not have obvious defects in hemostasis.[31-33] Despite this, counter-intuitively, both fXI and fXII deficient mice are as resistant to $FeCl_3$-induced thrombosis as are fIX deficient mice.[31-33] The findings have been corroborated using a variety of other models and methods for inducing thrombosis,[33-35] and clearly indicate that an anticoagulant (anti-hemostatic) phenotype is not a prerequisite for an antithrombotic effect, at least under experimental conditions. More recent work has shown that mice lacking PK[35-37] or HK[38] are also resistant to injury-induced thrombus formation. Taken as a whole, these data imply that a process similar to classic contact activation may be driving thrombus formation through the intrinsic pathway in the mouse models.

Studies in primates have also demonstrated roles for fXI and fXII in experimental thrombosis.[33,39-42] This work utilized inhibitory antibodies or antisense oligonucleotides (discussed below) to produce transient deficiency states. Thrombus formation was studied in prothrombotic collagen- or TF-coated vascular grafts inserted into temporary arteriovenous shunts in olive baboons (*Papio anubis*). Interestingly, in these studies the antithrombotic effect of fXI inhibition appeared to be greater than that of fXII inhibition.[33,40,41] A reduction in thrombus formation was detectable with as little as 50% reduction in plasma fXI level,[42] with the maximum effect achieved at ≤20% of normal plasma concentration.

While the data from the animal studies support the notion that the intrinsic pathway contributes to thrombosis, there are limitations to the preclinical analyses that must be considered. As the animal species tested do not have natural propensities to form arterial or venous thrombi in the same manner as humans, vessels must be injured in some manner to produce a thrombus. Injury-induced thrombosis in an otherwise healthy, and usually young, animal may not mimic formation of thrombi in the diseased vessels of older humans. The following section considers data from human populations on intrinsic pathway factors and thrombosis.

Factor IX and Factor VIII in Thrombosis in Humans

FIX is the precursor of the protease fIXa, and activated fVIII (fVIIIa) is its cofactor. The severe bleeding disorders associated with deficiency of fIX (hemophilia B) or fVIII (fVIII, hemophilia A) demonstrate their importance to hemostasis. There is a consistent corelation between plasma levels of fVIII in complex with von Willebrand factor (vWF) and risk for venous and arterial thrombosis in humans.[43-47] Supporting these observations, individuals with blood group O, who have 25-50% lower vWF and fVIII levels than individuals with a non-O blood group are at lower risk for venous and arterial thrombosis.[48] High plasma levels of fIX are associated with modestly increased risks for venous thromboembolism (VTE), myocardial infarction (MI) and stroke.[49-53] These findings are supported by the impression that hemophiliacs have a lower risk of arterial thrombosis than non-hemophiliacs.[43,54]

Factor XI in Thrombosis in Humans

For fXI levels, the strongest correlations are with risk for VTE and ischemic stroke. The 10% of subjects in the Leiden Thrombophilia Study (LETS) with the highest plasma fXI levels had a two-fold higher risk of VTE compared with the lower 90%,[55] a result supported by more recent data from the Longitudinal Investigation of Thromboembolism Etiology (LITE) cohort.[56] Consistent with these findings, fXI deficiency was associated with a reduced incidence of VTE in a study from Israel,[57] where severe deficiency is present in 1 in 450 individuals.[12,15] High plasma levels of fXI or fXIa were associated with increased risk for ischemic stroke in a study by Yang et al,[58] in the Atherosclerosis Risk in Communities (ARIC) study,[53] and in the Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study.[59] Similarly, severe fXI deficiency was associated with a reduced incidence of stroke.[60]

A role for fXI in MI is not as clear as for VTE or stroke. Plasma fXI levels correlated with MI risk in men in the Study of Myocardial Infarction Leiden (SMILE),[51] and were higher in women with coronary disease than in women without it in a group undergoing cardiac catheterization.[61] However, fXI was not a risk factor for MI in the ARIC[53] or RATIO[59] studies, and fXIa levels were not linked to coronary disease in the second Northwick Park Heart Study (NPHS-II).[62] The incidence of MI in 96 individuals with severe fXI deficiency was similar to the expected incidence for age-matched controls.[63] The data are in line with recent work by Siegerink *et al.* showing that an elevated level of fXIa correlated more closely with risk for ischemic stroke than for MI in young women,[64] and suggest that the contribution of fXI to thrombus formation is greater in some vascular beds than in others.

The Kallikrein-Kinin System in Thrombosis in Humans

Data supporting a role for fXII in VTE, stroke or MI in humans is weak, and there is insufficient information to assess PK and HK. It is worth noting that congenital deficiency of C1-Inhibitor (C1-INH), the major plasma regulator of fXIIa and α-kallikrein, causes the disorder hereditary angioedema, and does not appear to predispose to thrombosis.[26,27] FXII-deficient subjects are not protected from VTE,[65] and no differences in VTE incidences were noted across a range of fXII levels in LETS[66] and LITE[56] study participants. Data on fXII in arterial thrombosis are conflicting. FXII deficiency does not appear to protect individuals from stroke.[65] Plasma levels of fXIIa were inversely correlated with stroke risk in NPHS-II,[62] but were directly correlated with it in the RATIO study.[59] However, plasma fXII levels did not correlate with stroke risk in either study, nor in the ARIC study.[53] In NPHS-II elevated fXIIa measured by specific ELISA was associated with increased risk of MI, but fXIIa measured as a complex with C1-INH indicated the opposite effect.[62] In the RATIO study, fXIIa, fXII and PK levels were not associated with MI,[59] and risk of coronary events did not correlate with fXII in the ARIC study.[53] Finally, data from the SMILE cohort actually showed an inverse relationship between fXII levels and cardiovascular disease.[51] Endler *et al.* also noted an inverse association between fXII and death from ischemic heart disease, although the relationship did not hold for severe fXII deficiency (<10% of normal), where risk was comparable to that of the population mean.[67]

The Intrinsic Pathway and Thrombosis in Humans - Summary

While it is difficult to draw firm conclusions from the complicated human epidemiologic data presented above, it seems reasonable to conclude that the contribution of fXII to VTE, stroke, and MI in humans is probably smaller than for fXI and fIX. While this conclusion would conflict with data from mouse models, where fXII is a major contributor to thrombus formation, it is in reasonable agreement with results obtained in primates. It is possible that feedback activation of fXI by thrombin (**Figure 1B**) is relatively stronger in primates (including humans) than in mice, with fXIIa serving a smaller role in fXI activation. Another possibility is that a greater degree of fXIIa inhibition is required to produce an antithrombotic effect comparable to what is seen with fXIa inhibition in primates. These observations not withstanding, it is important to note that there are situations where fXIIa is very likely to be a major driver of thrombosis in humans. For example, contact activation is triggered when blood is exposed to artificial surfaces during cardiopulmonary bypass[68,69]

and extracorporeal membrane oxygenation (ECMO),[70] requiring full anticoagulation with heparin to prevent thrombotic complications.

Trials of Agents Targeting Components of the Intrinsic Pathway

Clinical Trials of Factor IXa inhibitors

FIX would appear to be an attractive target for antithrombotic therapy because of its role in sustaining thrombin generation, and its dual mode of activation through the extrinsic and intrinsic pathways. Patients with moderate or mild fIX deficiency (1-5% and 6-30% activity, respectively) generally experience bleeding only with trauma or surgery, suggesting subtotal fIXa inhibition may be tolerated reasonably well.[14] This hypothesis is supported by pre-clinical studies demonstrating potent antithrombotic effects for antibody, small molecule, and aptamer inhibitors of fIXa, with minimal bleeding.[71,72] However, the phenotype of complete fIX deficiency (severe hemophilia B), which includes spontaneous bleeding into joints and soft tissues,[14] indicates there will be limits to the intensity of therapeutic inhibition of fIXa. Human studies of fIXa inhibitors have been limited to testing the small molecule TTP889, and the RNA aptamer systems REG1and REG2.[73]

TTP889 is an orally available small molecule that partially inhibits fIXa activity (maximum ~90%).[73] In animal studies the drug had efficacy against venous and arterial thrombosis.[74] Interestingly, at what were considered supratherapeutic concentrations, TTP889, did not affect the aPTT. This contrasts with other types of fIXa inhibitors, which prolong the aPTT in animal models. TTP889 was tested in a randomized placebo controlled study as extended prophylaxis for VTE in patients who had undergone hip fracture repair followed by standard prophylaxis for 5 to 9 days.[72] Rates of VTE, as determined by venography were similar in the TTP889 and placebo groups after three weeks of therapy. This finding, and the fact that there were no differences in major bleeding, raised concerns that TTP889 was inadequately dosed. A phase II trial of TTP889 in patients undergoing ventricular assist device implantation was terminated early, and the drug has not been assessed further.

RNA aptamers are single stranded oligonucleotides that bind to a target of interest.[75] They are selected for specific functional capabilities from pools of random RNA sequences. Aptamers can be regulated with a complementary oligonucleotide that neutralizes activity through Watson-Crick base pairing. The REG1 and REG2 systems are comprised of parenterally administered aptamers that target fIXa.[73] REG1 includes the inhibitory aptamer RB006 (pegnivacogin) and its complement RB007 (anivamersen), both formulated for intravenous administration. REG2 is a formulation of RB006 for subcutaneous administration, with RB007 as its reversal agent. In phase 1 studies involving single and repeat escalating doses in healthy volunteers, RB006 demonstrated quick onset of action with significant fIXa inhibition.[76,77] While phase 2 studies demonstrated the feasibility of using the REG1 system in patients with coronary artery disease,[78,79] the RADAR phase 2b trial in patients with acute coronary syndrome was stopped early due to serious allergic reactions.[79] In a phase III trial of patients with acute coronary syndrome undergoing percutaneous coronary intervention, REG1 appeared to be comparable to bivalirudin in reducing incidence of ischemic events.[80] However the statistical power of the study was limited because it was stopped early, again because of allergic side effects. Therapy related

bleeding was also a concern. 0.4% of patients receiving REG1 had severe or fatal bleeding compared to 0.1% of patients receiving bivalirudin, and moderate to severe bleeding was also significantly higher in the REG1 group.[80]

The experience with fIXa inhibitors illustrates the challenge of producing an adequate antithrombotic effect by targeting a protease that serves a major role in hemostasis. The therapeutic window may be relatively narrow. The partial inhibition of fIXa achievable with TTP889 was inadequate to prevent thrombus formation in patients with coronary disease, while the greater inhibition achieved with REG1 lead to an increase in bleeding events compared with standard therapy. The limited data are far from sufficient to fully assess the utility of inhibiting fIXa as an antithrombotic strategy in humans. But what is available raises the possibility that it may be more difficult to achieve a reproducible effective and safe antithrombotic effect with a fIXa inhibitor than with inhibitors of fXa or thrombin.

Antisense-Induced Reduction of Factor XI

Modified DNA antisense oligonucleotides (ASOs) or “gapmers” have been widely used in research to specifically reduce expression of a protein of interest *in vivo*.[81] In contrast to the RNA aptamers described in the previous section, which directly inhibit coagulation in plasma, ASOs interfere with intracellular synthesis of a target protein. After entering a cell, the ASO binds through complementary base-pairing to a specific mRNA, leading to its selective degradation and reduced synthesis of the encoded protein. Second generation gapmers are avidly taken up by hepatocytes, facilitating targeting of coagulation factors. Their long tissue elimination half-lives allow administration at intervals of several days to weeks.

The ASO ISIS-416858 (now IONIS-416858) is complementary to a sequence in the human and rhesus macaque fXI mRNA.[82] In a phase I study, volunteers received three subcutaneous doses (50 to 300 mg) of ISIS-416858 during the first week of therapy, followed by weekly doses.[83] Those receiving 200 and 300 mg doses had, on average, ~80% reduction in plasma fXI, with >95% reduction achieved in some individuals. Plasma fXI levels remained well below baseline for several weeks after the last dose. There was no excessive bleeding, nor was there evidence of significant liver, kidney or blood cell abnormalities in study subjects. Mild irritation at injection sites was the most common side effect.

In a phase 2 randomized trial, ISIS-416858 was compared to standard dose low molecular weight heparin as prophylaxis to prevent VTE in patients undergoing knee replacement.[84] The ASO was given in 200 or 300 mg doses on study days 1, 3, 5, 8, 15, 22, and 29, with surgery on day 36. Additional doses were given the day of surgery, and three days post-surgery (day 39). Patients randomized to enoxaparin received the first dose the evening before surgery or 6 to 8 hours after surgery, and then once daily for at least eight days. On the day of surgery, average plasma fXI levels were 38% and 20% of normal in patients on the 200 or 300 mg ASO doses, respectively, and 93% in patients on enoxaparin. Several patients receiving the ASO had fXI levels <5% of normal. Bilateral lower extremity venography performed 8 to 12 days post-surgery detected thrombi in 30% of enoxaparin-treated patients, 27% of patients on the 200 mg ASO dose, and 4% on the 300 mg ASO

dose. The incidence of VTE was 5% in individuals from the two ASO groups with plasma fXI levels of ≤ 20% of normal at the time of surgery. There were few symptomatic clots in any treatment group (two in the 200 mg ASO group and one in the enoxaparin group). While there was no placebo group in this study, venographically detectable thrombi would be expected in ~45% of untreated patients undergoing knee replacement.[85] Clinically relevant bleeding occurred in 3% of patients on ASOs and 8% on enoxaparin.

There are several notable results from this study. First, 300 mg ISIS-416858 was superior to enoxaparin for reducing VTE. While the fVIIa/TF complex is thought to play a major role in VTE during orthopedic surgery,[86] this result raises the possibility that a fXI-dependent pathway dominates the process during knee replacement. Interestingly, thrombi that formed in the 300 mg ASO group were not only rarer, but considerably smaller, than thrombi in patients receiving 200 mg ASO or enoxaparin.[84] This dose-response mirrors results from primate studies with ISIS-416858 showing that reduction of fXI to 20% of normal is associated with a better antithrombotic effect than a reduction to 50% of normal.[42] Finally, because of the mechanism of action, ASO treatment was started five weeks before surgery, and patients were under the full drug effect during surgery. Despite this, abnormal hemostasis was not observed intra-operatively, even in patients with fXI levels <5% of normal, and post-operative bleeding was rare. This demonstrates the safety of fXI reduction in this setting, and supports the hypothesis that it is possible to dissociate an antithrombotic effect from a major effect on hemostasis, at least in some situations.

Targeting the kallikrein-kinin system

While protein (Ecallantide) and small molecule (BCX7353 and BCX4161) plasma kallikrein inhibitors have been used to treat idiopathic and hereditary angioedema, inhibition of fXIIa or α-kallikrein has not been tested in humans for treatment or prevention of thrombosis. Epidemiologic data does not support a role for fXII in common conditions such as VTE, stroke and MI; however, there are situations where contact activation likely contributes to thrombosis. As discussed, contact activation occurs during cardiopulmonary bypass[68,69] and ECMO,[70] where blood is exposed to artificial surfaces for prolonged periods. In a rabbit-ECMO model, the anti-fXIIa monoclonal IgG 3F7 was as effective as heparin in preventing thrombus formation in the extracorporeal circuit.[87,88] As expected, 3F7 did not compromise hemostasis, while heparin produced a significant bleeding propensity. Another potential advantage of a fXIIa inhibitor in this setting is that it would inhibit PK activation, blunting bradykinin production and reducing inflammation (**Figure 1C**). In rabbits in which thrombus formation is induced by intravenous placement of polyurethane catheters, ASO-induced depletion of fXII or fXI was more effective at maintaining vessel patency than fVII depletion.[89] Thus, inhibitors of fXIIa may be useful replacements or adjuncts for drugs such as heparin and warfarin in clinic scenarios in which blood is exposed to nonbiologic materials. A variety of compounds targeting fXIIa (reviewed in reference 90) are undergoing pre-clinical testing, and should be available for clinical testing in the near future.

Summary

The cascade-waterfall hypothesis of intrinsic blood coagulation has served as the foundation for tests used in clinical practice for more than fifty years.[10,11] While models based on this hypothesis, such as a the one shown in **Figure 1A**, accurately described the manner in which plasma clots in *in vitro* assays such as the aPTT, the clinical presentations of patients lacking various plasma factors involved in the cascade make it clear that the traditional intrinsic pathway is not an accurate representation of the processes that stop bleeding at a wound site. This has led to revisions, such as those shown in **Figure 1B**, that are in better agreement with clinical observations.[12,17] The bleeding tendencies seen in genetically altered mice lacking components of the plasma coagulation mechanism, in general, support the revised model, when it comes to hemostasis.[20] However, work over the past decade with animal models also points to the possibility that a process more similar to the classic intrinsic pathway contributes to pathologic thrombin generation. This raises the prospect that thrombosis might be treatable by targeting plasma factors that serve relatively minor roles in hemostasis. If therapies directed at proteases such as fXIa or fXIIa are effective at treating or preventing thrombosis, the safety profile of antithrombotic therapy could be substantially improved. The recent demonstration that ASO-mediated reduction of fXI is effective and safe for prevention of VTE in patients undergoing knee replacement surgery provides proof of concept for this premise.[84]

It is not certain why thrombus formation is dependent on fXI and fXII in some situations, but available data offer some clues. In the mouse and primate studies discussed in this chapter, inhibition of fXI or fXII did not prevent initial formation of clot on an injured vessel or thrombogenic surface, but had a dramatic effect on propagation of clot growth away from the vessel wall into the lumen.[31-34,39-41] Intraluminal thrombi forming in the absence of fXI or fXII are unstable, and fragment under the shear stresses in flowing blood. During hemostasis after vessel injury, clot forms primarily outside of the damaged blood vessel and within the blood vessel wall. Thrombus growth into the vessel lumen itself is probably not required to achieve hemostasis. The shear forces produced by flowing blood would be relatively high within the blood vessel lumen, and would increase as the lumen narrows from a growing intraluminal thrombus. Under these circumstances, the intrinsic pathway may support thrombin generation on the surface of the thrombus that maintains its stability and promotes its growth. We need to identify the factors that promote activation of intrinsic pathway proteases during thrombosis to better understand these processes.

A variety of compounds targeting fXI and fXIa have undergone pre-clinical testing and some have entered phase 1 studies.[90] Going forward, it seems reasonable to initially test fXI/fXIa inhibitors for prevention of thrombosis. At this point, there is insufficient information to predict if inhibition of intrinsic pathway components would be suitable for treatment of acute thrombotic processes. Based on the epidemiologic data, prevention of VTE and stroke should both be considered.[55-60] The results of the ASO knee replacement trial raise the possibility that fXIa inhibition is superior to current standard of care for preventing post-operative DVT.[84] Larger trials would be needed to conclusively demonstrate this, and to adequately assess differences in bleeding risk. The phase 2 ASO knee replacement trial was not powered sufficiently to evaluate bleeding.[84] Inhibition of FXI may be a particularly

attractive option for secondary VTE prevention. The strategy of extending anticoagulation therapy beyond the typical three to six months for treatment of an unprovoked deep vein thrombus or pulmonary embolus is being actively debated.[91,92] Prevention of rare fatal events by extending prophylaxis may be offset by the increased risk of major bleeding episodes. However, prevention of common complications of VTE that have a major impact on quality of life, such as post-thrombotic syndrome and pulmonary hypertension, may warrant extended treatment. The fXI ASO trials suggest that targeted fXI inhibition would be associated with a lower bleeding risk than with warfarin, or an oral thrombin or fXa inhibitor. Inhibiting fXIa may also be useful for primary or secondary prevention in patients with atrial fibrillation or other high-risk conditions who are not good candidates for conventional anticoagulation due to comorbidities, or as short-term prophylaxis after neurosurgery or other procedures where even modest anticoagulant-induced bleeding must be avoided.

Given the likely favorable safety profiles of inhibitors of fXIa and fXIIa, it may be possible to add such compounds to current standards of care to enhance therapeutic benefit without appreciably changing bleeding risk. FXI deficient patients have been treated safely with warfarin or anti-platelet therapy suggesting this approach would be safe.[93] It is also possible that fXIa or fXIIa inhibitors may allow doses of other antithrombotic drugs to be reduced, lowering overall bleeding risk. FXIa and fXIIa inhibitors may have an advantage over current anticoagulants in situations where contact activation is triggered by exposure of blood to artificial surfaces such as extracorporeal circuits and indwelling intravascular devices.[68-70,87-89] The recent work showing that FXIIa inhibition prevents thrombus formation in experimental ECMO[87,88] suggests that inhibitors of fXIIa or fXIa may be able to replacement or supplement heparin in patients on extracorporeal circuits. There is also a need for alternatives to warfarin for patients with mechanical heart valves. While the contributions of FXIa and FXIIa to thrombus formation induced by mechanical heart valves have not been evaluated, there is evidence that components of such valves can induce contact activation in plasma.[94]

With the demonstration that therapeutic manipulation of fXI can produce a potent antithrombotic effect in humans, future work should focus on establish the clinical scenarios in which fXI and fXII contribute to thrombosis, and determining the optimal target for each situation. If drugs directed at these proteases show efficacy in clinical trials, their safety profiles should widen the spectrum of conditions in which antithrombotic therapy can be administered, and increase the number of patients who are eligible for antithrombotic therapy.

Bibliography

1. Crawley JT, Zanardelli S, Chion CK, Lane DA. The central role of thrombin in hemostasis. J Thromb Haemost. 2007; 5(Suppl1):95–101. [PubMed: 17635715]
2. Yeh CH, Hogg K, Weitz JI. Overview of the new oral anticoagulants: opportunities and challenges. Arterioscler Thromb Vasc Biol. 2015; 35:1056–65. [PubMed: 25792448]
3. Mulloy B, Hogwood J, Gray E, et al. Pharmacology of heparin and related drugs. Pharmacol Rev. 2016; 68:76–141. [PubMed: 26672027]

4. Ageno W, Gallus AS, Wittkosky A, et al. Oral anticoagulation therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012; 141(Suppl2):e44S–e88S. [PubMed: 22315269]
5. Crowther MA, Warkentin TE. Bleeding risk and the management of bleeding complications in patients undergoing anticoagulant therapy: focus on new anticoagulant agents. Blood. 2008; 111:4871–9. [PubMed: 18309033]
6. Chai-Adisaksopha C, Crowther M, Isayama T, et al. The impact of bleeding complications in patients receiving target-specific oral anticoagulants: a systematic review and meta-analysis. Blood. 2014; 124:2450–8. [PubMed: 25150296]
7. Kenne E, Nickel KF, Long AT, et al. Factor XII: a novel target for safe prevention of thrombosis and inflammation. J Intern Med. 2015; 278:571–85. [PubMed: 26373901]
8. Chen Z, Seiffert D, Hawes B. Inhibition of factor XI activity as a promising antithrombotic strategy. Drug Discov Today. 2014; 19:1435–9. [PubMed: 24794465]
9. Gailani D, Bane CE, Gruber A. Factor XI and contact activation as targets for antithrombotic therapy. J Thromb Haemost. 2015; 13:1383–95. [PubMed: 25976012]
10. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. Nature. 1964; 202:498–9. [PubMed: 14167839]
11. Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood coagulation. Science. 1964; 145:1310–2. [PubMed: 14173416]
12. Gailani, D.; Neff, AT. Rare coagulation factor deficiencies.. In: Hoffman, R.; Benz, EJ.; Silberstein, LE.; Heslop, HE.; Weitz, JI.; Anastasi, J., editors. Hematology: basic principles and practice. 6th edition. Saunders-Elsevier; Philadelphia, PA: 2013. p. 1971-1986.
13. Wolberg AS, Mast AE. Tissue factor and factor VIIa – hemostasis and beyond. Thromb Res. May; 2012 129(Suppl2):S1–S4. [PubMed: 22417944]
14. Carcao, M.; Moorehead, P.; Lillicrap, D. Hemophilia A and B.. In: Hoffman, R.; Benz, EJ.; Silberstein, LE.; Heslop, HE.; Weitz, JI.; Anastasi, J., editors. Hematology: basic principles and practice. 6th edition. Saunders-Elsevier; Philadelphia, PA: 2013. p. 1940-1960.
15. Duga S, Salomon O. Congenital factor XI deficiency: an update. Semin Thromb Hemost. 2013; 39:621–31. [PubMed: 23929304]
16. James P, Salomon O, Mikovic D, et al. Rare bleeding disorders – bleeding assessment tools, laboratory aspects and phenotype and therapy of FXI deficiency. Haemophilia. 2014; 20(Suppl4): 71–5. [PubMed: 24762279]
17. Broze GJ. Tissue factor pathway inhibitor and the revised theory of coagulation. Annu Rev Med. 1995; 46:103–12. [PubMed: 7598447]
18. Mackman N. The role of tissue factor and factor VIIa in hemostasis. Anesth Analg. 2009; 108:1447–52. [PubMed: 19372318]
19. Doolittle RF. Step-by-step evolution of vertebrate coagulation. Cold Spring Harb Symp Quant Biol. 2009; 74:35–40. [PubMed: 19667012]
20. McManus, MP.; Gailani, D. Animal Models of Diseases: Translational Medicine Perspective for Drug Discovery and Development. Bentham Scientific Publishers; 2012. Mouse models of coagulation factor deficiencies; p. 67-121.
21. Ponczek MB, Gailani D, Doolittle RF. Evolution of the contact phase of vertebrate blood coagulation. J Thromb Haemost. 2008; 6:1876–83. [PubMed: 18761718]
22. Emsley J, McEwan PA, Gailani D. Structure and function of factor XI. Blood. 2010; 115:2569–77. [PubMed: 20110423]
23. Naito K, Fujikawa K. Activation of human blood coagulation factor XI independent of factor XII. Factor XI is activated by thrombin and factor XIa in the presence of negatively charged surfaces. J Biol Chem. 1991; 266:7353–8. [PubMed: 2019570]
24. Gailani D, Broze GJ Jr. Factor XI activation in a revised model of blood coagulation. Science. 1991; 253:909–12. [PubMed: 1652157]
25. Matafonov A, Sarilla S, Sun MF, et al. Activation of factor XI by products of prothrombin activation. Blood. 2011; 118:437–45. [PubMed: 21527525]

26. Long AT, Kenne E, Jung R, et al. Contact system revisited: an interface between inflammation, coagulation, and innate immunity. J Thromb Haemost. in press.
27. Schmaier AH. The contact activation and kallikrein/kinin systems: pathophysiologic and physiologic activities. J Thromb Haemost. 2016; 14:28–39. [PubMed: 26565070]
28. Frick IM, Björck L, Herwald H. The dual role of the contact system in bacterial infectious disease. Thromb Haemost. 2007; 98:497–502. [PubMed: 17849037]
29. Frick IM, Akesson P, Herwald H, et al. The contact system – a novel branch of innate immunity generating antibacterial peptides. EMBO J. 2006; 25:5569–78. [PubMed: 17093496]
30. Lin HF, Maeda N, Smithies O, et al. A coagulation factor IX-deficient mouse model for human hemophilia B. Blood. 1997; 90:3962–6. [PubMed: 9354664]
31. Wang X, Cheng Q, Xu L, et al. Effects of factor IX or factor XI deficiency on ferric chloride-induced carotid artery occlusion in mice. J Thromb Haemost. 2005; 3:695–702. [PubMed: 15733058]
32. Renné T, Pozgajová M, Grüner S, et al. Defective thrombus formation in mice lacking coagulation factor XII. J Exp Med. 2005; 202:271–81. [PubMed: 16009717]
33. Cheng Q, Tucker EI, Pine MS, et al. A role for factor XIIa-mediated factor XI activation in thrombus formation in vivo. Blood. 2010; 116:3981–9. [PubMed: 20634381]
34. Müller F, Mutch NJ, Schenk WA, et al. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. Cell. 2009; 139:1143–56. [PubMed: 20005807]
35. Revenko AS, Gao D, Crosby JR, et al. Selective depletion of plasma prekallikrein or coagulation factor XII inhibits thrombosis in mice without increased bleeding. Blood. 2011; 188:5302–11. [PubMed: 21821705]
36. Bird JE, Smith PL, Wang X, et al. Effects of plasma kallikrein deficiency on haemostasis and thrombosis in mice: murine ortholog of the Fletcher trait. Thromb Haemost. 2012; 107:1141–50. [PubMed: 22398951]
37. 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. [PubMed: 25339356]
38. Merkulov S, Zhang WM, Komar AA, et al. Deletion of murine kininogen gene 1 (mKng1) causes loss of plasma kininogen and delays thrombosis. Blood. 2008; 111:1274–81. [PubMed: 18000168]
39. Gruber A, Hanson SR. Factor XI-dependence of surface- and tissue factor-initiated thrombus propagation in primates. Blood. 2003; 102:953–955. [PubMed: 12689935]
40. Tucker EI, Marzec UM, White TC, et al. Prevention of vascular graft occlusion and thrombus-associated thrombin generation by inhibition of factor XI. Blood. 2009; 113:936–44. [PubMed: 18945968]
41. Matafonov A, Leung PY, Gailani AE, et al. Factor XII inhibition reduces thrombus formation in a primate thrombosis model. Blood. 2014; 123:1739–46. [PubMed: 24408325]
42. Crosby JR, Marzec U, Revenko AS, et al. Antithrombotic effect of antisense factor XI oligonucleotide treatment in primates. Arterioscler Thromb Vasc Biol. 2013; 33:1670–8. [PubMed: 23559626]
43. Lowe G. Factor IX and deep vein thrombosis. Haematologica. 2009; 94:615–617. [PubMed: 19407317]
44. Bertina RM. Elevated clotting factor levels and venous thrombosis. Pathophysiol Haemost Thromb. 2003; 33:395–400. [PubMed: 15692250]
45. Rumley A, Lowe GDO, Sweetnam PM, et al. Factor VIII, von Willebrand factor and the risk of major ischaemic heart disease in the Caerphilly Study. Br J Haematol. 1999; 105:110–6. [PubMed: 10233372]
46. Whincup P, Danesh J, Walker M, et al. Von Willebrand factor and coronary heart disease: prospective study and meta-analysis. Europ Heart J. 2002; 23:1764–70.
47. Tsai AW, Cushman M, Rosamond WD, et al. Coagulation factors, inflammation markers, and venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE). Am J Med. 2002; 113:636–42. [PubMed: 12505113]

48. Wu O, Bayoumi N, Vickers MA, et al. ABO (H) blood groups and vascular disease: a systematic review and meta-analysis. J Thromb Haemost. 2008; 6:62–9. [PubMed: 17973651]
49. van Hylckama Vlieg A, van der Linden IK, Bertina RM, et al. High levels of factor IX increase the risk of venous thrombosis. Blood. 2000; 95:3678–82. [PubMed: 10845896]
50. Heikal NM, Murphy KK, Crist RA, et al. Elevated factor IX activity is associated with an increased odds ratio for both arterial and venous thrombotic events. Am J Clin Pathol. 2013; 140:680–5. [PubMed: 24124147]
51. Doggen CJ, Rosendaal FR, Meijers JC. Levels of intrinsic coagulation factors and the risk of myocardial infarction among men: Opposite and synergistic effects of factors XI and XII. Blood. 2006; 108:4045–51. [PubMed: 16931632]
52. Tanis B, Algra A, van der Graaf Y, et al. Procoagulant factors and the risk of myocardial infarction in young women. Eur Heart J. 2006; 77:67–73.
53. Suri MF, Yamagishi K, Aleksic N, et al. Novel hemostatic factor levels and risk of ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. Cerebrovasc Dis. 2010; 29:497–502. [PubMed: 20299790]
54. Sramek A, Kriek M, Rosendaal FR. Decreased mortality of ischaemic heart disease among carriers of haemophilia. Lancet. 2003; 362:351–4. [PubMed: 12907007]
55. Meijers JC, Tekelenburg WL, Bouma BN, et al. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med. 2000; 342:696–701. [PubMed: 10706899]
56. Cushman M, O'Meara ES, Folsom AR, et al. Coagulation factors IX through XIII and the risk of future venous thrombosis: the longitudinal investigation of thromboembolism etiology. Blood. 2009; 114:2878–83. [PubMed: 19617576]
57. Salomon O, Steinberg DM, Zucker M, et al. Patients with severe factor XI deficiency have a reduced incidence of deep-vein thrombosis. Thromb Haemost. 2011; 105:269–73. [PubMed: 21057700]
58. Yang DT, Flanders MM, Kim H, et al. Elevated factor XI activity levels are associated with an increased odds ratio for cerebrovascular events. Am J Clin Pathol. 2006; 126:411–15. [PubMed: 16880142]
59. Siegerink B, Govers-Riemslag JW, Rosendaal FR, et al. Intrinsic coagulation activation and the risk of arterial thrombosis in young women: results from the Risk of Arterial Thrombosis in relation to Oral contraceptives (RATIO) case-control study. Circulation. 2010; 122:1854–61. [PubMed: 20956210]
60. Salomon O, Steinberg DM, Koren-Morag N, et al. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. Blood. 2008; 111:4113–7. [PubMed: 18268095]
61. Berliner JI, Rybicki AC, Kaplan RC, et al. Elevated levels of Factor XI are associated with cardiovascular disease in women. Thromb Res. 2002; 107:55–60. [PubMed: 12413590]
62. Govers-Riemslag JW, Smid M, Cooper JA, et al. The plasma kallikrein-kinin system and risk of cardiovascular disease in men. J Thromb Haemost. 2007; 5:1896–903. [PubMed: 17723129]
63. Salomon O, Steinberg DM, Dardik R, et al. Inherited factor XI deficiency confers no protection against acute myocardial infarction. J Thromb Haemost. 2003; 1:658–61. [PubMed: 12871398]
64. Siegerink B, Maino A, Algra A, et al. Hypercoagulability and the risk of myocardial infarction and ischemic stroke in young woman. J Thromb Haemost. 2015; 13:1568–75. [PubMed: 26178535]
65. Zeerleder S, Schloesser M, Redondo M. Reevaluation of the incidence of thromboembolic complications in congenital factor XII deficiency--a study on 73 subjects from 14 Swiss families. Thromb Haemost. 1999; 82:1240–6. [PubMed: 10544906]
66. Koster T, Rosendaal FR, Briet E, et al. John Hageman's factor and deep-vein thrombosis: Leiden Thrombophilia Study. Brit J Haematol. 1994; 87:422–4. [PubMed: 7947293]
67. Endler G, Marsik C, Jilma B, et al. Evidence of a U-shaped association between factor XII activity and overall survival. J Thromb Haemost. 2007; 5:1143–8. [PubMed: 17388965]
68. Sonntag J, Dähnert I, Stiller B, et al. Complement and contact activation during cardiovascular operations in infants. Ann Thorac Surg. 1998; 65:525–31. [PubMed: 9485258]
69. Wendel HP, Jones DW, Gallimore MJ. FXII levels, FXIIa-like activities and kallikrein activities in normal subjects and patients undergoing cardiac surgery. Immunopharmacology. 1999; 45:141–4. [PubMed: 10615003]

70. Plötz FB, van Oeveren W, Bartlett RH, et al. Blood activation during neonatal extracorporeal life support. J Thorac Cardiovasc Surg. 1993; 105:823–32. [PubMed: 7683735]
71. Howard EL, Becker KC, Rusconi CP, et al. Factor IXa inhibitors as novel anticoagulants. Arterioscler Thromb Vasc Biol. 2007; 27:722–7. [PubMed: 17272750]
72. Eriksson BI, Dahl OE, Lassen MR, et al. Partial factor IXa inhibition with TTP889 for prevention of venous thromboembolism: an exploratory study. J Thromb Haemost. 2008; 6:457–63. [PubMed: 18088349]
73. Eikelboom JW, Zelenkofske SL, Rusconi CP. Coagulation factor IXa as a target for treatment and prophylaxis of venous thromboembolism. Arterioscler Thromb Vasc Biol. 2010; 30:382–7. [PubMed: 20139356]
74. Rothlein R, Shen JM, Naser N, et al. TTP889, a novel orally active partial inhibitor of FIXa inhibits clotting in two a/v shunt models without prolonging bleeding times. Blood. 2005; 106:A1886.
75. Woodruff RS, Sullenger BA. Modulation of the coagulation cascade using aptamers. Arterioscler Thromb Vasc Biol. 2015; 35:2083–91. [PubMed: 26315404]
76. Dyke CK, Steinhubl SR, Kleiman NS, et al. First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase 1a pharmacodynamic evaluation of a drug-antidote pair for the controlled regulation of factor IXa activity. Circulation. 2006; 114:2490–7. [PubMed: 17101847]
77. Chan MY, Rusconi CP, Alexander JH, et al. A randomized, repeat-dose, pharmacodynamic and safety study of an antidote-controlled factor IXa inhibitor. J Thromb Haemost. 2008; 6:789–96. [PubMed: 18284597]
78. Cohen MG, Purdy DA, Rossi JS, et al. First clinical application of an actively reversible direct factor IXa inhibitor as an anticoagulation strategy in patients undergoing percutaneous coronary intervention. Circulation. 2010; 122:614–22. [PubMed: 20660806]
79. Povsic TJ, Vavalle JP, Aberle LH, et al. A Phase 2, randomized, partially blinded, active-controlled study assessing the efficacy and safety of variable anticoagulation reversal using the REG1 system in patients with acute coronary syndromes: results of the RADAR trial. Eur Heart J. 2013; 34:2481–9. [PubMed: 22859796]
80. Lincoff AM, Mehran R, Povsic TJ, et al. Effect of the REG1 anticoagulation system versus bivalirudin on outcomes after percutaneous coronary intervention (REGULATE-PCI): a randomized clinical trial. Lancet. 2015 in press.
81. Zhang H, Löwenberg EC, Crosby JR, et al. Inhibition of the intrinsic coagulation pathway factor XI by antisense oligonucleotides: a novel antithrombotic strategy with lowered bleeding risk. Blood. 2010; 116:4684–92. [PubMed: 20807891]
82. Younis HS, Crosby J, Huh JI, et al. Antisense inhibition of coagulation factor XI prolongs APTT without increased bleeding risk in cynomolgus monkeys. Blood. 2012; 119:2401–8. [PubMed: 22246038]
83. Liu Q, Bethune C, Dessouki E, et al. ISIS-FXI$_{Rx}$, A Novel and Specific Antisense Inhibitor of Factor XI, Caused Significant Reduction in FXI Antigen and Activity and Increased aPTT without Causing Bleeding in Healthy Volunteers. Blood. 2011; 118:A209.
84. Büller HR, Bethune C, Bhanot S, et al. Factor XI antisense oligonucleotide for prevention of venous thrombosis. N Engl J Med. 2015; 372:232–40. [PubMed: 25482425]
85. Fuji T, Fujita S, Tachibana S, et al. A dose-ranging study evaluating the oral factor Xa inhibitor edoxaban for the prevention of venous thromboembolism in patients undergoing total knee arthroplasty. J Thromb Haemost. 2010; 8:2458–68. [PubMed: 20723033]
86. Owens AP 3rd, Mackman N. Tissue factor and thrombosis: The clot starts here. Thromb Haemost. 2010; 104:432–9. [PubMed: 20539911]
87. 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.
88. Worm M, Köhler EC, Panda R, et al. The factor XIIa blocking antibody 3F7: a safe anticoagulant with anti-inflammatory activities. Ann Transl Med. 2015; 3:247. [PubMed: 26605293]

89. Yau JW, Liao P, Fredenburgh JC, et al. Selective depletion of factor XI or factor XII with antisense oligonucleotides attenuates catheter thrombosis in rabbits. Blood. 2014; 123:2102–7. [PubMed: 24501216]

90. Gailani D. Future prospects for contact factors as therapeutic targets. Hematology Am Soc Hematol Educ Program. 2014; 2014:52–9. [PubMed: 25696834]

91. Schulman S. Secondary prevention of venous thromboembolism. BMJ. 2013; 347:f5440. [PubMed: 24018259]

92. Kearson C, Akl EA. Duration of anticoagulant therapy for deep vein thrombosis and pulmonary embolism. Blood. 2013; 123:1794–801.

93. Salomon O, Seligsohn U. New Observations on factor XI deficiency. Haemophilia. 2004; 10(Suppl4):184–7. [PubMed: 15479396]

94. Jaffer IH, Fredenburgh JC, Hirsh J, Weitz JI. Medical device-induced thrombosis: what causes it and how can we prevent it? J Thromb Haemost. 2015; 13(Suppl1):S72–81. [PubMed: 26149053]

KEY POINTS

- The term *intrinsic pathway* refers to a series of sequential reactions involving the plasma proteins factors VIII, IX, XI and XII; and pre-kallikrein and high molecular weight kininogen that are required for initiation of coagulation in the activated partial thromboplastin time (aPTT) assay.
- Certain components of the intrinsic pathway that serve a limited role in hemostasis (factor XI), or are not required for hemostasis (factor XII, prekallikrein and high molecular weight kininogen) are required for clot formation in animal models of thrombosis.
- Epidemiologic data indicate that factor XI contributes to venous thromboembolism and ischemic stroke, and may contribute to myocardial infarction in humans, while factor XII likely contributes to thrombus formation when blood is exposed to artificial surfaces, such as during cardiopulmonary bypass and extracorporeal membrane oxygenation.
- Reducing factor XI to 20% of normal by antisense oligonucleotide technology was more effective than standard dose low molecular weight heparin in preventing venous thrombosis during knee replacement surgery, without comprising intraoperative or post-operative hemostasis.
- By targeting components of the intrinsic pathway of coagulation with therapeutic inhibitors, it may be possible to uncouple antithrombotic effects from anticoagulant (antihemostatic) effects, improving the safety of antithrombotic therapy.

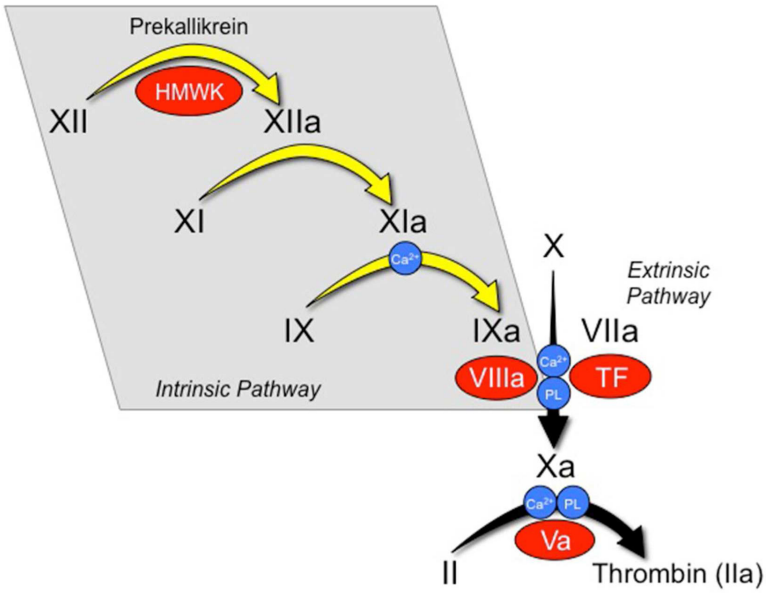

Figure 1. The waterfall / cascade model of coagulation

Shown are the proteolytic reactions leading to thrombin generation during plasma coagulation initiated through the intrinsic (yellow arrows) and extrinsic (factor VIIa/TF) pathways. The zymogen (precursor) forms of plasma trypsin-like proteases involved in coagulation are represented by the black Roman numerals, and their active protease forms by Roman numerals followed by a lowercase “a”. Prekallikrein is the zymogen of the protease α-kallikrein. Protein cofactors are shown in red circles, and reactions requiring calcium (Ca2+) or phospholipid (PL) are indicated in blue. A feature of this model is that factor Xa and thrombin formation can be initiated through two distinct pathways. In the prothrombin time (PT) assay, coagulation is initiated by addition of the cofactor tissue factor (TF) to plasma. TF in complex with factor VIIa forms the extrinsic pathway of coagulation, which activates factor X to Xa. In the activated partial thromboplastin time (aPTT) assay, addition of a negatively charged substance (usually a purified earth) to plasma triggers activation of factor XII, and sets off the series of enzymatic reactions referred to as the intrinsic pathway of coagulation. The intrinsic pathway protease factor IXa is an activator of factor X in this model, bypassing the need for factor VIIa/TF. Factor Xa converts prothrombin to the protease thrombin, which then induces coagulation of plasma by converting fibrinogen to fibrin.

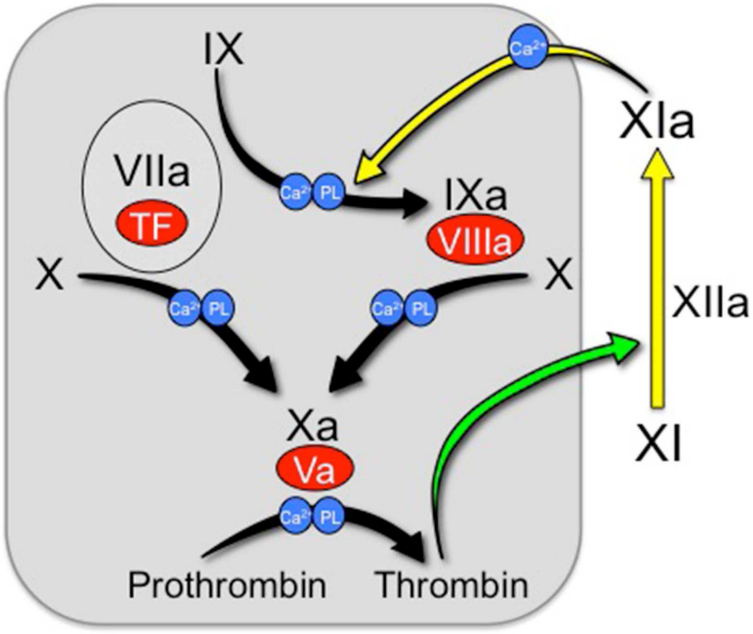

Figure 2. Factor VIIa/TF-initiated model of coagulation

The model presents our current understanding of the major plasma protease-substrate interactions during thrombin generation *in vivo*. The nomenclature and symbols are the same as those used in **Figure 1**. Coagulation is initiated by exposure of the factor VIIa/TF complex to blood, leading to the activation of factors IX and X. Early in the process, factor Xa converts prothrombin to thrombin to initiate coagulation, while factor IXa generates more factor Xa to sustain the process. In this scheme, factor XIa may contribute to thrombin generation by converting additional factor IX to factor IXa. As factor XII deficiency does not cause a hemostatic abnormality, it is thought that factor XI is converted to factor XIa by a protease other than factor XII. For example, fXI can be activated by thrombin (green arrow). In contrast to the cascade/waterfall model in **Figure 1**, here the intrinsic pathway (yellow arrows) triggered by initial factor VIIa/TF-mediated thrombin generation is not part of mechanism for initiating coagulation, but functions to sustain factor IX activation, and ultimately thrombin generation, to maintain the integrity of a clot over time.

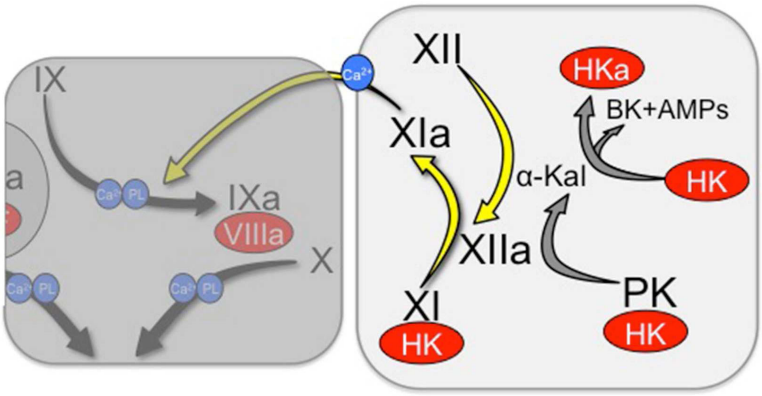

Figure 3. The kallikrein-kinin (contact activation) system

When blood is exposed to a variety of surfaces and natural compounds (often with a net negative charge), the plasma zymogens factor XII and prekallikrein (PK) bind to the surface and reciprocally activate each other. Binding of PK is facilitated by the cofactor high molecular weight kininogen (HK). The process is probably triggered by traces of factor XIIa or α-kallikrein present in plasma. α-kallikrein can cleave HK to release the proinflammatory peptide bradykinin. The kallikrein-kinin may have several functions, including contributing to the innate immune response to microorganism invasion. While factor XIIa can initiate thrombin generation through the intrinsic pathway of coagulation (yellow arrows) by activating factor XI, the kallikrein-kinin system does not appear to be required for hemostasis. Humans and other animals lacking factor XII, PK or HK do not have a bleeding disorder. However, there is mounting evidence that contact activation-induced coagulation through the intrinsic pathway may contribute to growth of pathologic clots during thrombosis. The nomenclature and symbols are the same as those used in **Figure 1**.