Plasma kallikrein (PK) is a plasma protease that drives procoagulant- and proinflammatory reactions via the intrinsic pathway of coagulation and the kallikrein-kinin system, respectively. The structure and biochemistry of PK have been analysed in great detail; however, its in vivo functions are just beginning to emerge. In this issue of Thrombosis and Haemostasis, Bird et al. presents a mouse model with target ablation of the PK gene (1). Severe deficiency of PK abolishes experimental thrombosis in arterial and venous vascular beds. Despite the striking thromboprotective effects conferred by targeting the protease, PK deficiency has minor effect on the haemostatic capacity of gene-deficient mice. This animal model provides a promising tool to study the pathogenetic role of the protease in models of thrombosis and inflammation with exciting therapeutic perspectives.

In the original waterfall cascade model of plasma coagulation, fibrin formation may be initiated by two distinct pathways, triggered either by exposure of blood to tissue factor at an injured vessel wall or to blood-borne (intrinsic) factors. The intrinsic pathway of coagulation is initiated by factor (F)XII, in a reaction involving high-molecular-weight kininogen (HK) and PK, collectively referred to as the plasma contact system (2). Contact with negatively charged surfaces such as kaolin or polyphosphate activates FXII, and activated FXII (FXIIa) cleaves PK to generate active kallikrein, which in turn reciprocally activates FXII zymogen. FXIIa initiates fibrin formation through its principal substrate of the intrinsic coagulation pathway, FXI, and also triggers liberation of the potent inflammatory mediator bradykinin (BK) by PK-mediated HK cleavage (3). Binding of BK to the kinin B2 receptor (B2R) causes an immediate nicotinic oxide-mediated vasodilation through an increase of cGMP in vascular smooth muscle cells, activates various pro-inflammatory signalling pathways that dilate vessels, induces chemotaxis of neutrophils and increases vascular permeability and fluid efflux (4). In a positive feedback loop, kallikrein also activates surface-bound FXII to FXIIa. Furthermore, PK has the capacity to convert pro-urokinase to urokinase, plasminogen to plasmin, and for triggering the renin-angiotensin system. Due to its BK-forming capacity, kallikrein has potent in vitro effects on leukocyte migration, stimulates monocytes, and induces neutrophil aggregation. Recent studies have suggested a role of PK for control of intracerebral haemorrhage in hyperglycaemia (5). In contrast, hereditary deficiencies in PK (Fletcher trait) and its substrate HK (Fitzgerald trait) were not associated with pathology, such as abnormal bleeding, despite causing a partial thromboplastin time (aPTT). The contact surface used in aPTT assays is typically a negatively charged non-physiological substance such as kaolin (aluminum silicate), celite (diatomaceous earth), or ellagic acid. The aPTT is widely used in clinical practice for preoperative screening and monitoring of anticoagulant therapy. Despite its important role in fibrin formation in the aPTT assay, PK-driven coagulation does not appear to have haemostatic or other physiological functions in vivo. This notion is based on the observation that PK deficiency, a rare incidentally observed coagulation abnormality in humans, has not yet been associated with pathology, such as abnormal bleeding, despite causing a marked prolongation of the aPTT. Similar to PK-deficient individuals, humans lacking the other contact proteins, FXII or HK, do not have impaired haemostasis.

Recently, FXII-deficient (FXII-/-) mice were generated and phenotyped to study the function of coagulation FXII in vivo (9, 10). Similar to FXII-deficient humans, FXII-/- mice have a normal haemostatic capacity as assessed by a tail-bleeding assay. Completely unexpected, intravital fluorescence microscopy and blood flow measurements in three distinct arterial beds revealed a severe defect in FXII-defi-
cient mice in thrombus formation induced by different methods of injuries (11). The data from studies of FXII-deficient mice challenge the “revised model of coagulation” and demonstrate a crucial role of FXII for pathological thrombin generation and coagulation activation in vivo. Mice lacking FXI or treated with an inhibitor that blocks FXI activation by FXIIa are similarly protected from occlusive thrombus formation without haemostasis impairment, suggesting that FXII impacts only on pathologic clotting via the intrinsic pathway (12, 13). Despite the striking protective effect in these models, FXII-deficient mice, like their human counterparts, do not have spontaneous or injury-related haemorrhage.

Consistent with a critical role of contact system proteins in thrombosis, HK-deficient mice also have an antithrombotic phenotype. The mouse has two kininogen genes (kininogen-1 and -2), which account for about 50% of kininogen plasma concentration each. Targeted inactivation of kininogen-1 was sufficient to prolong the aPTT and to impair thrombus formation in the carotic artery induced by the Rose Bengal photothrombosis model (14).

These results demonstrate that contact system-driven fibrin formation is specifically important for pathologic thrombus formation but has no function for fibrin formation during normal haemostasis. This raises the possibility that targeting PK may offer a strategy for prevention or treatment of thrombosis that is not associated with the high rate of haemorrhage that accompanies currently used anticoagulants (15).

The importance of the contact system for thrombosis is not restricted to animal models. Proteins of the contact system are highly conserved between mice and humans, and results from animal models can easily be transferred to human disease states, e.g. FXI appears to participate in thrombosis in humans similarly to its role in mouse and primate models (16). Apart from some case reports that describe the association of PK deficiency and thrombosis, no prospectively designed study has been conducted to determine the risk of either bleeding or thromboembolic events in PK deficient individuals (17). Together the new data from contact system-deficient mice and patients challenge the concept that pathological thrombus formation represents a dysregulation of normal haemostatic mechanisms and suggest that the dogma of the coagulation balance is in need for revision.

Current anticoagulation therapy is based primarily on heparins, vitamin K antagonists, or indirect inhibitors of FXa, like the synthetic pentasaccharide fondaparinux sodium. The need for regular monitoring and dose adjustments for some of these drugs (unfractionated heparin and warfarin), or for parenteral administration (heparins, fondaparinux) is inconvenient. Furthermore, use of these drugs is associated with an increased risk of life-threatening bleeding. New oral anticoagulants with lower, but still significant, bleeding risk, including direct inhibitors of thrombin (e.g. dabigatran etexilate) or FXa (e.g. rivaroxaban), have entered the market. The anti-thrombotic effects and bleeding risks for these drugs have been assessed in several clinical trials (18). Bleeding is an expected consequence with these compounds because they all target proteins that are critical components of the haemostatic mechanism. In contrast, the experimental data of Bird et al. and existing information on the limited roles of PK, FXI and FXII in haemostasis suggest that pharmaceutical targeting of these proteases could provide antithrombotic benefits without an increase in the incidence of major bleeds.

Conflicts of interest
None declared.

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