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 nitric 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 haemostatic abnormalities (6). The serpin C1 esterase inhibitor (C1INH) is the major plasma inhibitor of PK and controls enzymatic activity of the protease.

Plasma prokallikrein (PPK) is the zymogen of the trypsin-like serine protease PK. PPK is expressed by hepatocytes in humans and mice and circulates in plasma at a concentration of 50 g/ml, predominantly in a noncovalent 1:1-complex with HK. PK is a paralog of FXI, and both genes have evolved from a common ancestor (7). The principal mechanism of PK is to cleave the high-molecular-weight precursor (HK) that liberates BK. Despite similar names, PK is not related to the large group of kallikrein of the trypsin-like serine protease domain (8). However, in contrast to human FXI, human PK is a monomer. PK migrates as a doublet with 86- and 88-kDa on non-reducing SDS-polyacrylamide gels. The two bands represent differentially glycosylated proteins.

Contact activation is the mechanism of contact-induced FXII zymogen activation. Contact activation provides the mechanistic basis for one of the most commonly used diagnostic clotting test the activated 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 elagic 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 pathologial 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 phototrombosis 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 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 Benga

Conflicts of interest

None declared.

This editorial reflects the view of its author(s) and is not representative of the view of the Editorial Board or the Publishers.

References