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Physiologic Activities of the Contact Activation System

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Abstract

The plasma contact activation (CAS) and kallikrein/kinin (KKS) systems consist of 4 proteins: factor XII, prekallikrein, high molecular weight kininogen, and the bradykinin B2 receptor. Murine genetic deletion of factor XII (*F12*$^{-/-}$), prekallikrein (*Klkb1*$^{-/-}$), high molecular weight kininogen (*Kgn1*$^{-/-}$) and the bradykinin B2 receptor (*Bdkrb2*$^{-/-}$) yield animals protected from thrombosis. With possible exception of *F12*$^{-/-}$ and *Kgn1*$^{-/-}$ mice, the mechanism(s) for thrombosis protection is not reduced contact activation. *Bdkrb2*$^{-/-}$ mice are best characterized and they are protected from thrombosis through over expression of components of the renin angiotensin system (RAS) leading to elevated prostacyclin with vascular and platelet inhibition. Alternatively, prolylcarboxypeptidase, a PK activator and degrader of angiotensin II, when deficient in the mouse leads to a prothrombotic state. Its mechanism for increased thrombosis also is mediated in part by components of the RAS. These observations suggest that thrombosis in mice of the CAS and KKS are mediated in part through the RAS and independent of reduced contact activation.

The plasma contact activation system (CAS) is a group of proteins [factor XII (XII), prekallikrein (PK), high molecular weight kininogen (HK)] that influence surface-activated blood coagulation tests [the activated partial thromboplastin time, activated clotting time (ACT)] but not hemostasis. XII and to a lesser extent PK influence these assays because they autoactivate into enzymes when incubated with biologic or artificial surfaces. In plasma, XII autoactivates on surfaces and activates PK to plasma kallikrein; plasma kallikrein activates XII and they then reciprocally amplify each other's activation. HK accelerates this process (Fig 1). Contact activation occurs at the interaction of blood with artificial surfaces such as thrombus on catheter tips, blood and platelet activation in cardiopulmonary bypass, or after adulteration of intravenous preparations (e.g. albumin, immunoglobulin or heparin) with the plasma kallikrein, activated forms of XII (Hageman factor fragment, βFXIIa), or a glysoaminoglycans like chondroitin sulfate. Additionally, several medical disorders such as sepsis, acute attacks of hereditary angioedema due to C1 inhibitor deficiency or mutated XII, adult respiratory distress syndrome (ARDS), and

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allergic reactions have contact activation with plasma kallikrein formation and bradykinin (BK) liberation.

A "physiologic process" is one consistent with normal functioning of an organism. Contact activation *in vivo* always arises under pathophysiologic (disease) circumstances. Contact activation in catheter thrombosis, cardiopulmonary bypass, sepsis, and ARDS leads to thrombin formation (Fig 1). Alternatively, contact activation after infusion of adulterated intravenous preparations, acute attacks of hereditary angioedema, or allergic reactions leads to tissue swelling and hypotension without thrombin formation. Activation of plasma PK by activated XII or prolylcarboxypeptidase (PRCP) (see below) results in plasma kallikrein cleaving HK to liberate BK (1,2). This pathway is the plasma kallikrein/kinin system (KKS) and it generates BK both physiologically and in disease states (Fig 1A).

We have shown that XII binds to endothelial cell uPAR, gC1qR, and cytokeratin 1, and, through uPAR, signals through β_1 integrins and EGFR to phosphorylate ERK1/2 and $AktS^{473}$ (3,4). XII binding results in cell growth and angiogenesis. *F12*$^{-/-}$ mice have reduced angiogenesis in wound repair and ischemia-reperfusion (unpublished) (4). HK also binds platelets, neutrophils, endothelial cells via uPAR, gC1qR, and cytokeratin 1, is contained in platelet alpha granules and endothelial cells (EC), and is a receptor for PK and factor XI (XI) on EC (5–14). PK and XI bind EC with and without HK (13,14). HK is anti-proliferative, anti-angiogenic, and pro-apoptotic. Deficiencies in HK, XII, and PK (unpublished) are associated with reduced plasma BK, although XII deficiency is associated with half normal plasma BK levels whereas HK and PK deletions are associated with virtually no BK (15,16). BK mediates its activities through constitutive bradykinin B2 receptor (B2R, *Bdkrb2*) and the B1 receptor (B1R, *Bdkrb1*) that arises in inflammatory states. BK stimulation of its receptors results in tPA liberation, NO formation, and PGI_2 production (Fig 1A).

The CAS and KKS have an intimate relationship with the renin-angiotensin system (RAS) (Fig 1). The crosstalk between KKS and RAS is profound (17). Kininase II, the major enzyme that degrades BK, is angiotensin converting enzyme (ACE) (Fig 1A). ACE degrades BK to make bradykinin-(1–5) [BK-(1–5), (RPPGF)] that is a low affinity direct thrombin inhibitor (18). ACE also creates angiotensin II (AngII). AngII binds to the angiotensin receptor 1 (AT1R) to stimulate vasoconstriction, blood pressure elevation, and increased tissue factor (TF) and PAI-1 release from endothelium (Fig 1A). AngII also binds the angiotensin receptor 2 (AT2R) to produce NO and PGI_2. The binding affinity of AngII to the AT1R vs AT2R is the same so the receptor that has higher expression has the dominant effect. The B2R regulates expression of the AT1R and AT2R by formation of heterodimeric complexes (19). Angiotensin-1–7) [Ang-1–7)] which is the angiotensin converting enzyme 2 (ACE2) or PRCP breakdown product of AngII, binds to the receptor Mas (20). Mas activation by Ang-1–7) also elevates tPA, NO and PGI_2 (Fig 1A).

Recently several laboratories have recognized a variety of biologic substances, such as exposed vessel collagen, DNA and RNA, aggregated proteins, and long chain polyphosphates, that serve as platforms for XII autoactivation. Further, XII deficient mice (*F12*$^{-/-}$) were recognized to have reduced surface activation-induced pulmonary embolism

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(collagen/epinephrine and long chain polyphosphate) (21). In an inferior vena cava venous stasis model, *F12*$^{-/-}$ mice also have reduced thrombosis (22). These findings suggest a novel notion that the CAS is involved in thrombosis.

Our laboratory has focused on the contributions of the CAS and KKS on arterial thrombosis. Four animals models of the CAS-KKS with reduced BK delivery to tissues have been examined: the XII KO (*F12*$^{-/-}$), the kininogen I KO (*Kgn*$^{-/-}$*),* BK B2 receptor KO (*Bdkrb2*$^{-/-}$), and PK (*Klkb1*$^{-/-}$) mice (15,21–25). In each case, gene deletion is associated with arterial thrombosis protection. With exception of the *Bdkrb2*$^{-/-}$ mice, the mechanism(s) for thrombosis protection has not been elucidated in detail.

The finding that *Bdkrb2*$^{-/-}$ mice are protected from thrombosis was not intuitive since BK has been known to stimulate endothelial cell NO, prostacyclin (PGI_2) formation and tissue plasminogen activator (tPA) liberation (23,24). *Bdkrb2*$^{-/-}$ mice have elevated BK and its ACE metabolite, BK-(1-5), due to less uptake and metabolism by the absent B2R (Fig 1B). BK-(1-5) is elevated because of increased ACE activity that also yields elevated AngII. Usually, AngII stimulates the angiotensin receptor 1 (AT1R) to elevate blood pressure and increase thrombosis risk. However, if the angiotensin receptor 2 (AT2R) is elevated, AngII would bind to the AT2R to produce NO and PGI_2. When examined, we found increased AT2R, NO, and PGI_2 in *Bdkrb2*$^{-/-}$ mice (23). Treatment of *Bdkrb2*$^{-/-}$ with PD123314, an AT2R antagonist, L-NAME, an eNOS antagonist, or nimesulide, a COX2 inhibitor, corrected the thrombosis protection in these animals.

In addition to elevated AngII, we also found elevated angiotensin-(1–7) [Ang-(1–7)] in *Bdkrb2*$^{-/-}$ mice (Fig 1B). Ang-(1–7) is the prolylcarboxypeptidase (PRCP) or ACE2 breakdown product of AngII (20). Further, the Ang-(1–7) receptor, Mas, was increased as well. Stimulation of the Mas receptor also increases NO and PGI_2. In *Bdkrb2*$^{-/-}$ *mice,* the Mas antagonist A-779 corrected the prolonged thrombosis time to normal. Thus, in *Bdkrb2*$^{-/-}$ mice, thrombosis protection is produced by a double increase in AngII and Ang-(1–7) working on increased receptors AT2R and Mas (24).

How do the elevated NO and PGI_2 precisely influence thrombosis risk? *Bdkrb2* mice have long bleeding times. PGI_2, not NO, was mostly responsible for the observed platelet defect (24). *Bdkrb2*$^{-/-}$ mice have 3-fold PGI_2 elevation, elevated cGMP and cAMP, but normal thrombin- and ADP-induced $\alpha_{2b}\beta_3$ integrin complex formation and P-selectin expression and fibrinogen binding, respectively. *Bdkrb2*$^{-/-}$ mice also have an integrin-mediated spreading defect on fibrinogen and collagen and a GPVI activation defect to convulxin, CRP, and collagen (24). The platelet defect is host dependent, not intrinsic to their platelets because on bone marrow transplantation of normal bone marrow into *Bdkrb2*$^{-/-}$ mice, the normal platelets acquire the same platelet function defect (24). To date, we have not examined the influence of NO and PGI_2 on vascular function in *Bdkrb2*$^{-/-}$ mice, but postulate that it has additional mechanisms for arterial thrombosis protection. Investigations with the PK KO (*Klkb1*$^{-/-}$) mice indicate a related mechanism conferring thrombosis protection by influence on vascular function (unpublished). *Kgn1*$^{-/-}$ mice are deficient in BK because kininogen is the parent protein for BK (15) but as yet, there are no mechanistic data on these animals.

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Alterations between the RAS and KKS also lead to higher thrombosis risk. Prolylcarboxypeptidase (PRCP) is a membrane serine protease that degrades biologically active peptides with C-terminus Pro-X bonds and activates PK (1,26,27). Its substrates include αMSH_{1-13} metabolism regulating central anorexia and AngII metabolism leading to the production of Ang-(1–7) (20,28). In kidney, PRCP is as an important producer of Ang-(1–7) as ACE2 (20,24). PRCP gene trap ($PRCP^{gt/gt}$) mice are lean, hypertensive, and prothrombotic (29). In PRCP deficiency, there is increased plasma PK and reduced XII (29). Further, there is reduced renal Ang-(1–7) production (Fig 1C) (44). $PRCP^{gt/gt}$ mice have increased vessel ROS with reduced eNOS, uncoupled eNOS, reduced protein C activation due to reduced thrombomodulin expression, increased vascular tissue factor and plasminogen activator inhibitor (59). These animals also have reduced cell growth, angiogenesis, wound injury repair, ischemia/reperfusion repair, and increased arterial neointima/media growth after endothelial cell denudation (30). PRCP's influence on vascular well-being has nothing to do with contact activation.

In sum, we propose that the proteins of the plasma CAS and KKS have profound effects on thrombosis. With possible exception of $F12^{-/-}$ and $Kgn1^{-/-}$ mice, the regulation of thrombosis risk is not through reduced contact activation leading to thrombin formation, but rather through interactions with the RAS. As we become more knowledgeable in the mechanism(s) by which each of the proteins of the plasma CAS and KKS influence thrombosis risk *in vivo*, the contributions of RAS are becoming more evident. Finally, the hypothesis for contact activation induced thrombosis is 50 years old this year. Although we are learning new things about contact activation, it is not the only way by which the proteins of the CAS influence thrombosis risk. It is time to think outside the box for new mechanistic pathways.

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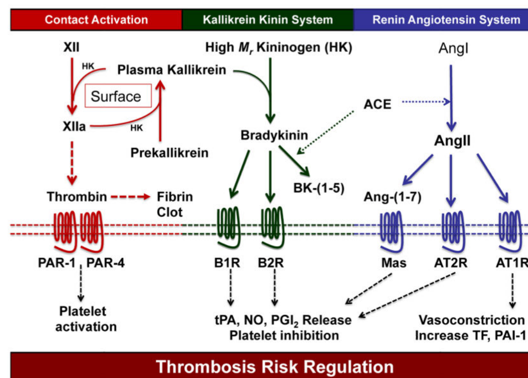

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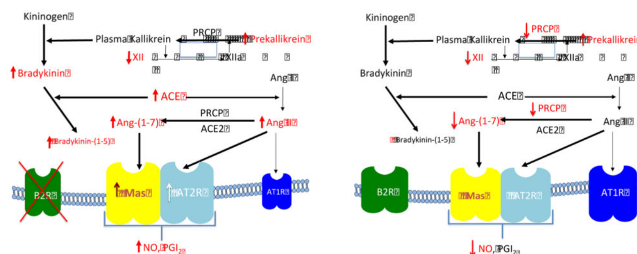

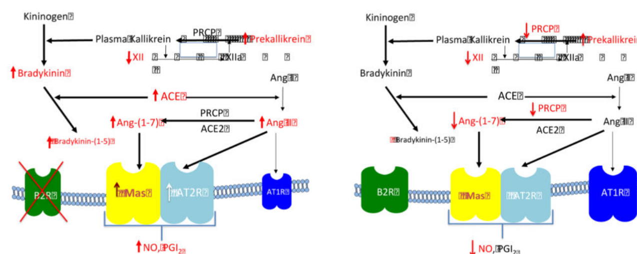

Figure 1.

Panel A Juxtaposition of the plasma contact activation system with the plasma kallikrein kinin and renin angiotensin systems. XII=factor XII; HK or High M_r Kininogen=high molecular weight kininogen; XIIa=activated factor XII; PAR-1=protease activated receptor 1; PAR-4=protease activated receptor 4; BK-(1-5)=bradykinin-(1-5); B1R=bradykinin B1 receptor; B2R=bradykinin B2 receptor; tPA=tissue plasminogen activator; NO=nitrous oxide; PGI_2=prostacyclin; ACE=angiotensin converting enzyme; Ang1=angiotensin I; AngII=angiotensin II; Ang-(1–7)=angiotensin-(1–7); Mas: a receptor for Ang-(1–7); AT2R=angiotensin receptor 2; AT1R=angiotensin receptor 1; TF=tissue factor; PAI-1=plasminogen activator inhibitor-1. See text for explanation of the diagram. **Panel B**: Changes in the plasma KKS and RAS in bradykinin B2 receptor deleted (*Bdkrb2*$^{-/-}$) mice that influence thrombosis risk. In *Bdkrb2*$^{-/-}$ mice there is increased plasma PK and decreased XII. Further there is elevation of BK, bradykinin-(1-5), ACE activity, AngII, and Ang-(1–7). The elevated AngII and Ang-(1–7) stimulate elevated receptors AT2R and Mas to produced increase NO and PGI_2. These latter entities inhibit platelet activation and influence vasculature to create an animal that is protected from arterial thrombosis. All abbreviations in this figure are identical to those in Fig. 1A. **Panel C**: Changes in the plasma KKS and RAS in prolylcarboxypeptidase (PRCP) gene trap mice (*PRCP*$^{gt/gt}$) mice that influence thrombosis risk. In *PRCP*$^{gt/gt}$, there is reduced XII and increased PK. Additionally there is reduced PK activation and reduced Ang-(1–7) formation. Vascular and cultured endothelial studies indicate that there is reduced and uncoupled eNOS (59). These animals have hypertension and arterial thrombosis. Their vasculature shows inflammation with increased vascular reactive oxygen species associated with reduced and uncoupled eNOS, reduced and dysfunctional thrombomodulin, increased tissue factor, and increased plasminogen activator inhibitor.