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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.

Introduction

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 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

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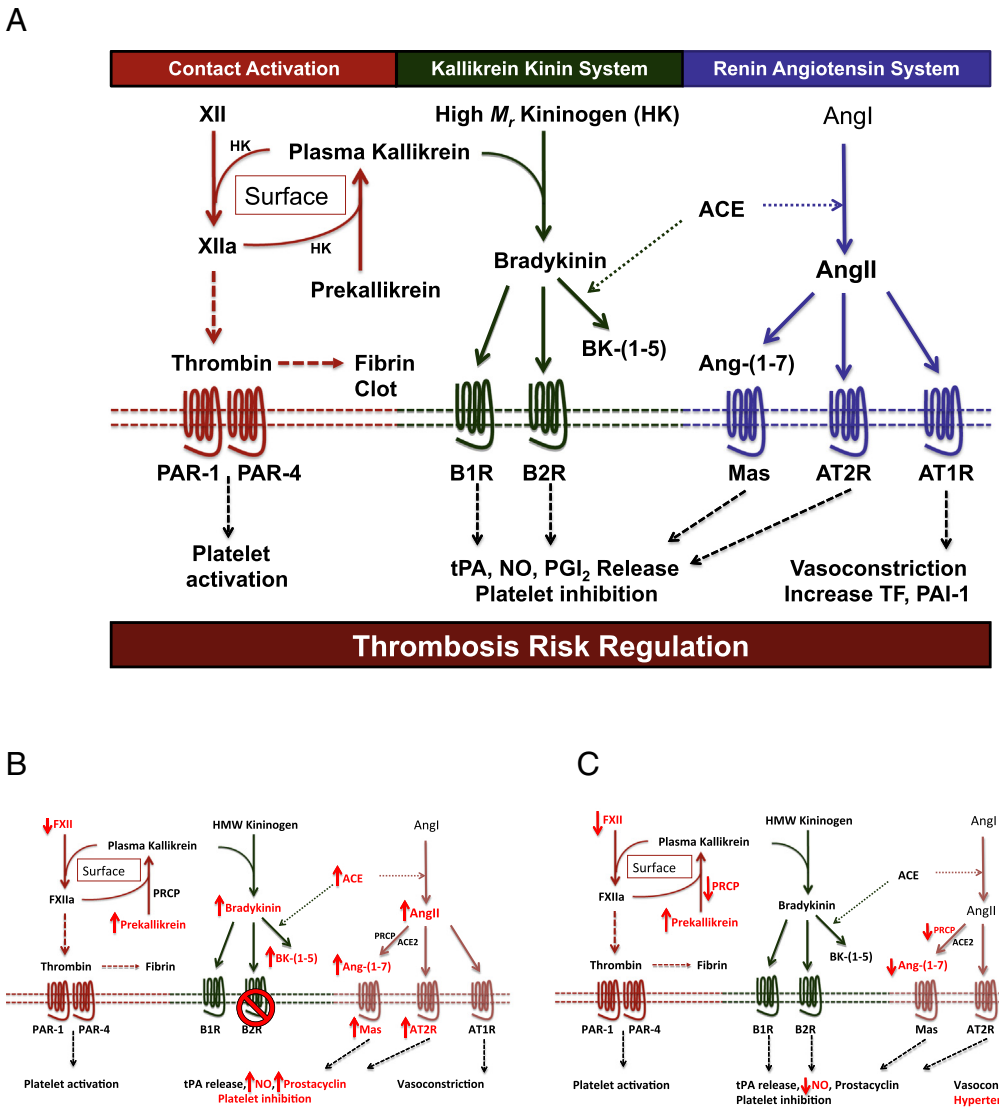

Fig. 1. Panel A: Juxtaposition of the plasma contact activation system with the plasma kallikrein kinin and renin angiotensin systems. XII = factor XII; HK or High M_r Kininogen = high molecular weight kininogen; XIIa = activated factor XII; PAR-1 = protease activated receptor 1; PAR-4 = protease activated 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 [29]. 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.

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, 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 (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