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The regulatory role of coagulation factors in vascular function

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1. ABSTRACT

The coagulation takes place in the hemostasis system and is a is hallmarked by a complex interplay of reactions between coagulation proteins. In the presence of a vascular breach, the conversion of prothrombin to thrombin leads to the formation of insoluble fibrin fibers that will stop bleeding and limit blood loss. Hemostasis is known to be disturbed in many diseases leading to hemorrhages or thrombosis. Despite the role of coagulation in hemostasis, recent evidences suggested that coagulation factors are involved in other (patho)physiological processes in the vasculature not necessarily marked by overt clotting, such as atherosclerosis and hypertension. Many direct (through protease activated receptors) or indirect effects of several coagulation factors are now well described. This review is focusing on the role of coagulation factors in the (dys)regulation of vascular function.

2. INTRODUCTION

The coagulation takes place in the physiological system of hemostasis. In case of vascular injury, formation of a clot in concert with platelet activation will stop bleeding and limit blood loss (1). The aftermath of hemostasis, fibrinolysis corresponds to wound healing and resolution of thrombotic material. Hemostasis is known to be disturbed in many diseases leading to hemorrhages or thrombosis (2). Proper coagulation needs the presence of calcium, phospholipids, and cellular receptors to allow several zymogens to be activated in a cascade and in feedback loops (3). Despite the role of coagulation in hemostasis, recent evidence suggest that coagulation factors are involved in other physiological as well as pathological processes such as the regulation of vascular function.

2.1. From tissue factor to fibrin clot

The coagulation system is a complex interplay of reactions between coagulation proteins leading to the conversion of prothrombin to thrombin and the formation of insoluble fibrin fibers. Most of the coagulation factors are synthesized by the liver, the endothelium and/or myeloid cells and consist of inactive zymogens.

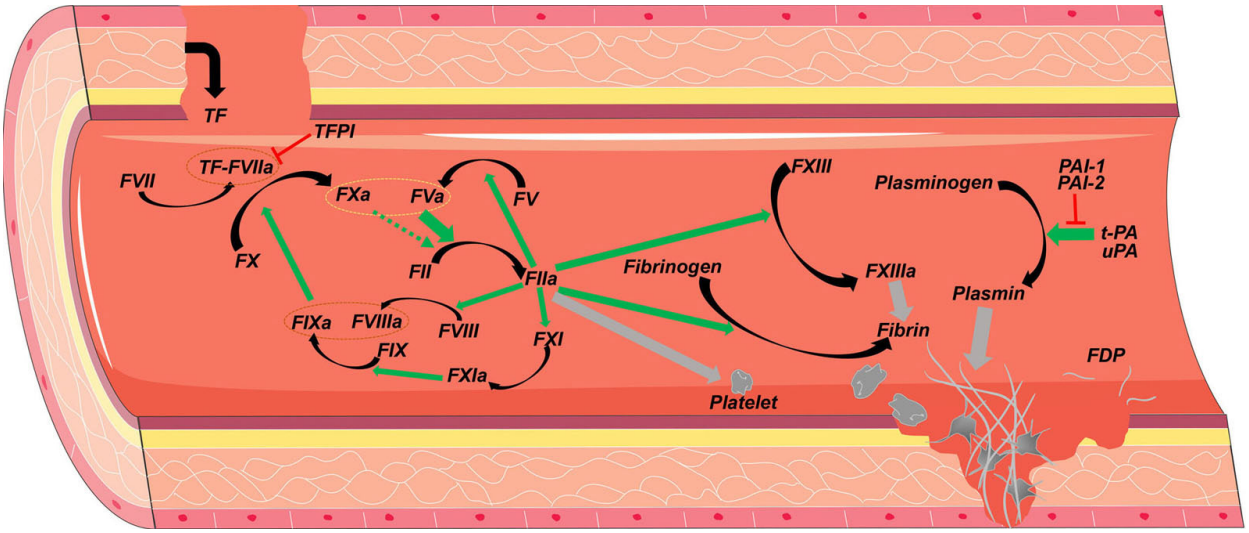

Figure 1. Overview of the coagulation cascade. In the presence of a vascular injury the subendothelium releases tissue factor (TF), which will lead to factor VII activation (FVIIa) and to factor X activation (FXa). The initially formed FXa can convert prothrombin (FII) to thrombin (FIIa) and FIIa will amplify its own formation through activation of factor V (FVa), factor VIII (FVIIIa) and factor XI (FXIa). FXIa can activate factor IX (FIXa), which will activate FX with the help of FVIIIa. FIIa cleaves fibrinogen, leading to the formation of insoluble fibrin and the fibrin clot is stabilized with activated factor XIII (FXIIIa). Lysis of the clot is made possible by the conversion of plasminogen to plasmin via tissue plasminogen activator (t-PA) and urokinase plasminogen activator (uPA), leading to the formation of fibrin degradation products. Fibrinolysis can be inhibited by plasminogen activator inhibitors (PAI-1, PAI-2). Black curved arrows: "conversion to"; green arrows: "activation of" red T-bar: "inhibition of"; grey arrows: additional pleiotropic effects.

The coagulation cascade is traditionally separated into two pathways, the intrinsic and extrinsic pathways. Despite this historical separation, many interactions exist between the coagulation factors of these two pathways. Tissue factor (TF) is the first factor from the extrinsic pathway (also named tissue factor pathway). It is made of three domains, one extracellular constituted of two fibronectin type-III domains, a transmembrane domain and a cytosolic domain (4,5). This membrane bound factor is present in the subendothelial layers and is exposed to the blood in case of injury. In the presence of vessel damage, exposed TF will bind to FVII and in the presence of calcium this complex will lead to the activation of FVII (FVIIa). This complex, called extrinsic Xase is able to activate FX to FXa. FXa will form a complex with FVa called "prothrombinase complex". FXa from this complex will cleave two sites of prothrombin (FII) leading to the formation of active thrombin (FIIa) (6). Prothrombin is a vitamin K dependent zymogen able to bind to phospholipids through its gamma-carboxyglutamic (Gla) domain in presence of calcium. Once the first molecules of thrombin are formed, several processes occur (Figure 1)

- Autoamplification of thrombin formation through activation of FV, FVIII and FXI by thombin
- Formation of the first fibrin fibers
- Activation of the inducible anticoagulant systems. Formed thrombin will bind to thrombomodulin present on the surface of endothelial cells (ECs) and lead to a thousand-fold increased speed of protein C (PC) activation (activated protein C, APC) (7). Endothelial protein C receptor (EPCR) can also increase the conversion speed of PC to APC. In turn, APC will inactivate FVa and FVIIIa, thus shutting down the prothrombinase and the intrinsic Xase complex.

2.2. The intrinsic pathway

FXII is the initiator of the intrinsic pathway. This pathway is also called contact phase since FXII can be activated by negatively changed surfaces like platelet polyphosphates or extracellular RNA (8,9). This little amount of FXIIa will activate kallikrein. In return, kallikrein can activate FXII (10). Activation of FXII initiates the intrinsic pathway by activation of FXI that will activate FIX. FIXa can form the so-called "intrinsic Xase" complex with FVIIIa and promote thrombin formation (11,12). Thrombin can autoamplify its activation via its ability to activate FXI (13). Contrary to FXI deficiency (hemophilia C) which may be associated with a mild bleeding phenotype, FXII deficiency do not lead to abnormal bleeding indicating that FXII is not essential for hemostasis (14–16). Concerning the thrombotic risk related to the coagulation factors of the intrinsic pathway, elevated FXI, FIX and FVIII are associated with increased risk of venous thromboembolisms (17–19).

2.3. Fibrin clot formation and fibrinolysis

Fibrinogen consists of 3 chains (Aα, Bβ, γ) assemble into dimers (Aα γ and Bβ γ) and then into

hexamers (Aα, Bβ, γ)2 to form two Aα-Bβ-γ trimers linked by disulfide bonds. This soluble glycoprotein can be cleaved by thrombin (on α and β chains) and form fibrin molecules that possess the ability to polymerize. Fibrin polymers are cross-linked with the help of the transglutaminase FXIIIa, to form a fibrin clot (20).

Fibrinolysis, the pathway counteracting coagulation, results in the degradation of fibrin, which is also an important step in thrombus resolution. The main molecule responsible for thrombus resolution is plasmin. Similar to other coagulation factors, it is released as an inactive zymogen, plasminogen. In circulating blood plasminogen cannot be converted to active plasmin; this is only possible in case of a conformational change occurring once plasminogen is bound to thrombotic material or cell surfaces (21). Once plasminogen has adopted an open conformation, it can be cleaved by tissue plasminogen activator (t-PA) or urokinase plasminogen activator (uPA), kallikrein, FXIIa or FXIa (22). Plasmin not only cleaves fibrin but also fibronectin, thrombospondin, laminin or von Willebrand factor (VWF). Action of plasmin can be inhibited by plasminogen activator inhibitors-1 and -2 (PAI-1 and PAI-2), which will inhibit plasminogen activation *via* tPA and uPA inhibition.

2.4. Coagulation profile in hypertension and other diseases related to vascular dysfunction

Essential hypertension represents the most common cardiovascular risk factor present in more than 20% of the adult population (23). Untreated hypertension can lead to many adverse outcomes such as renal failure, heart failure, atrial fibrillation and stroke. Hypertension increases levels of factor VII (FVII), fibrinogen and D-dimer suggesting the presence of a hypercoagulable state (24–26). On the anticoagulation side, antithrombin (AT) and PC were also found to be increased in hypertensive individuals. In rats with deoxycorticosterone-induced hypertension, thrombin-antithrombin complex levels (TAT), indicating increased thrombin generation *in vivo,* were increased (27). In this model, tissue factor (TF), which triggers the extrinsic coagulation pathway was increased and thrombomodulin (TM) which triggers the anticoagulant PC pathway was lowered. In spontaneously hypertensive rats, prothrombin and fibrinogen were increased while anticoagulant AT was also increased (28).

Hypertension is the most important risk factor for heart failure (HF) (29). In this disease, the risk of venous and arterial thrombosis is increased (30). Left ventricular dysfunction is an outcome of myocardial infarction (31,32). In the V-HeFT study, thromboembolism risk was poorly associated with left ventricle ejection fraction (33). On the contrary, the 1997 SAVE study presented an 18 % increased risk for every 5 % decrease of the left ventricle ejection fraction (34).

Atherosclerosis is commonly associated to vascular dysfunction. It develops when the vessel lumen narrows due to the formation of atheroma plaques mainly composed of immune cells generating foam cells and stiffening arterial vessels. Atherothrombosis occurs after rupture of an atherosclerotic lesion. Platelets adhere to the exposed subendothelial matrix molecules and VWF followed with strong coagulation activation (35). In atherosclerosis TF and FVII are expressed on macrophages and vascular smooth muscle cells (VSMCs) in the arterial wall and atherosclerotic plaques (36,37). FX was also found to be colocalized with macrophages in the plaque (38). In the same work the procoagulant state of advanced-stage atherosclerotic plaques was increased compared to early-stage plaques with elevated activities of TF, prothrombin, FX, FXII and increased thrombin generation and thrombin-antithrombin complex. Moreover, fibrin degradation products (D-dimer) are increased and associated with an increased risk of severe atherosclerosis (39).

Most of the studies previously cited observed thrombotic risk as an adverse effect of the vascular disease. Almost 20 years ago the concept was raised that heart failure might also change several aspects of the hemostatic system (40). Contractility reduction associated with cardiac chambers dilatation increases blood stasis, which in turn participates to thrombus formation. A significant number of patients who died from left ventricular aneurysm presented signs of thrombosis (30).

ECs are a major source of anticoagulant factors. These cells cover the inner face of blood vessels and avoid contact between blood and procoagulant surfaces, such as collagen and VSMCs, preventing clotting. Endothelial function is known to be altered in many vascular diseases. Hypertensive patients present increased circulating levels of VWF, normally stored in the Weibel-Palade bodies of ECs or the platelet α-granula. VWF factor is increased in HF where endothelial dysfunction is also well described (41–43). Other markers of endothelial activation, such as EPCR, are increased in hypertension (44). Finally, the synthesis of all coagulation factors is modified indicating profound changes in hemostasis biology and regulation (45,46).

3. COAGULATION FACTORS IN VASCULAR FUNCTION

3.1. Tissue factor: beyond hemostasis

TF triggers the extrinsic pathway of the coagulation cascade. It is expressed in the vascular

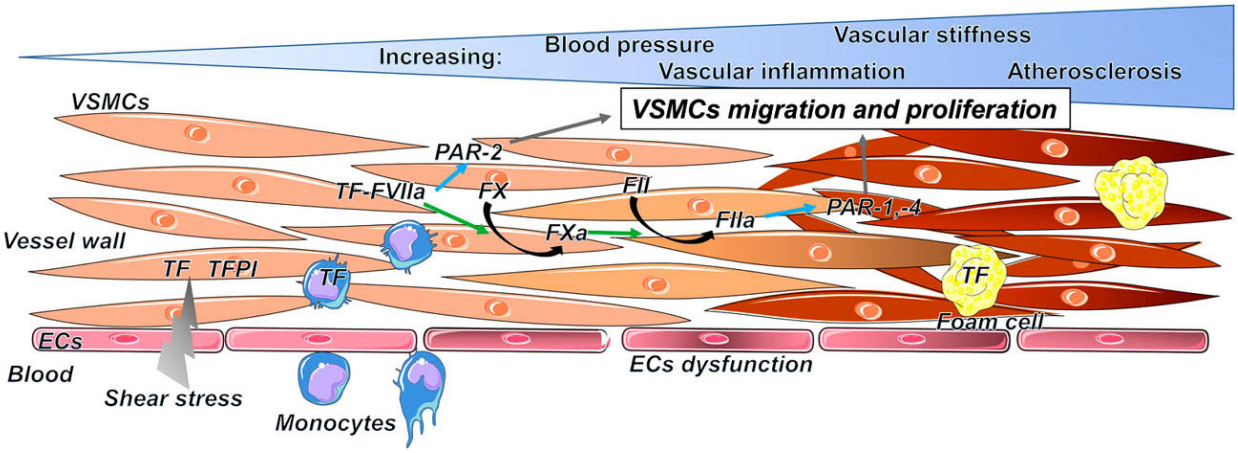

Figure 2. Implication of coagulation factors in the development of vascular dysfunction. Shear stress stimulates synthesis of tissue factor (TF) and TF pathway inhibitor (TFPI) by vascular smooth muscle cells (VSMCs). Monocytes crossing the endothelial cells (ECs) barrier can also produce TF. TF bound to FVIIa as well as generated thrombin (FIIa) can activate protease activated receptors (PAR-2, -1, -4), known to trigger migration and proliferation of VSMCs. Black curved arrows: "conversion to"; green arrows: "activation of"; blue arrows: receptor activation; grey arrows: additional pleiotropic effects. FII: prothrombin.

wall by VSMCs, ECs and fibroblasts, but also by myeloid cells such as macrophages and neutrophils (47). In the blood, monocytes are able to synthetize TF (48). This encrypted TF represent about 80 % of the TF expressed by monocytes and activation of these cells can lead to TF decrypting (49). Calcium ionophore, phosphatidylserine and also monocytes interaction with other cells or circulating microvesicles could also be implicated in the activation process (50–52).

Over the years, immune cells have gained recognition to be the main source of TF implicated in venous thrombosis and atherothrombotic events (53,54). Moreover, TF-rich microvesicles can be generated by monocytes through the ADP/P2X7 pathways and are well described to be procoagulant (55,56). Deletion of TF in monocytes and neutrophils prevents initiation of deep vein thrombosis in mouse models (57,58). Contrary to other coagulation factors, no TF deficiency was described in humans, and mice lacking TF are embryonically lethal (59,60). A disorganization of the yolk sac vasculature was observed in the TF deficient embryos highlighting the idea that TF was not only important for hemostasis but also for vessel development (61). In the 2000s, TF-FVIIa complex was found to be able to activate protease activated receptor 2 (PAR-2) and contributes to several processes such as angiogenesis, inflammation or cancer development (Figure 2) (62,63). Indeed, inhibition of TF-FVIIa complex had antiangiogenic properties in PAR-2-dependent neovascularization in hypoxia (64,65). Migration and proliferation of VSMCs can be modulated by TF-FVIIa through PAR-2 activation via ERK phosphorylation (66,67). More recently, FVIIa integrin-binding site was demonstrated to be required for integrin β1 complex formation leading to proangiogenic signaling independent of the TF-PAR-2 proangiogenic signaling (68).

VSMCs, as well as, ECs respond to shear stress, blood pressure, and pulse waves. Cyclic stretch created by blood pulsatility applied to VSMCs leads to vessel wall thinning and triggers intracellular signaling via mechanoreceptors like integrins, tyrosine kinase receptors or ion channels (69). In endothelial cells, shear stress attenuates tumor necrosis factor alpha (TNFα)-induced TF expression (70). TF pathway inhibitor (TFPI), a direct inhibitor of TF, is also modulated by cyclic stretch. A 10% mechanic stretch at 1 Hz leads to increased synthesis of TFPI by VSCMs (71). Interestingly, this relation between TFPI and increased pulse pressure and aortic stiffness was present in a cohort of postmenopausal woman. TFPI could be implicated in atherothrombosis via its ability to induce apoptosis in VSMCs (72). Moreover TFPI can also inhibit ECs proliferation thanks to its ability to recognize the very low density lipoprotein receptor (73).

3.2. PARs: the effectors of coagulation factor's cellular effects

PARs are a subfamily of the seven-transmembrane G-protein-coupled receptor superfamily. Four PARs are described and known to be expressed in platelets and vascular cells such as ECs and VSMCs (74). The distribution differs depending on cell types and species. For example, human platelets express PAR-1 and PAR-4 that mediates platelet activation while mice platelet express PAR-3 and PAR-4 (75,76).

PARs activation by proteases consists in the unmasking of an N-terminal "tethered ligand" (TL), which stays linked to the rest of the protein (77). In case of activation, the TL part of the receptor binds to the extracellular receptor domain leading to conformational changes and cellular signaling (78). This activation occurs in PAR-1, PAR-2 and PAR-4 while PAR-3 acts

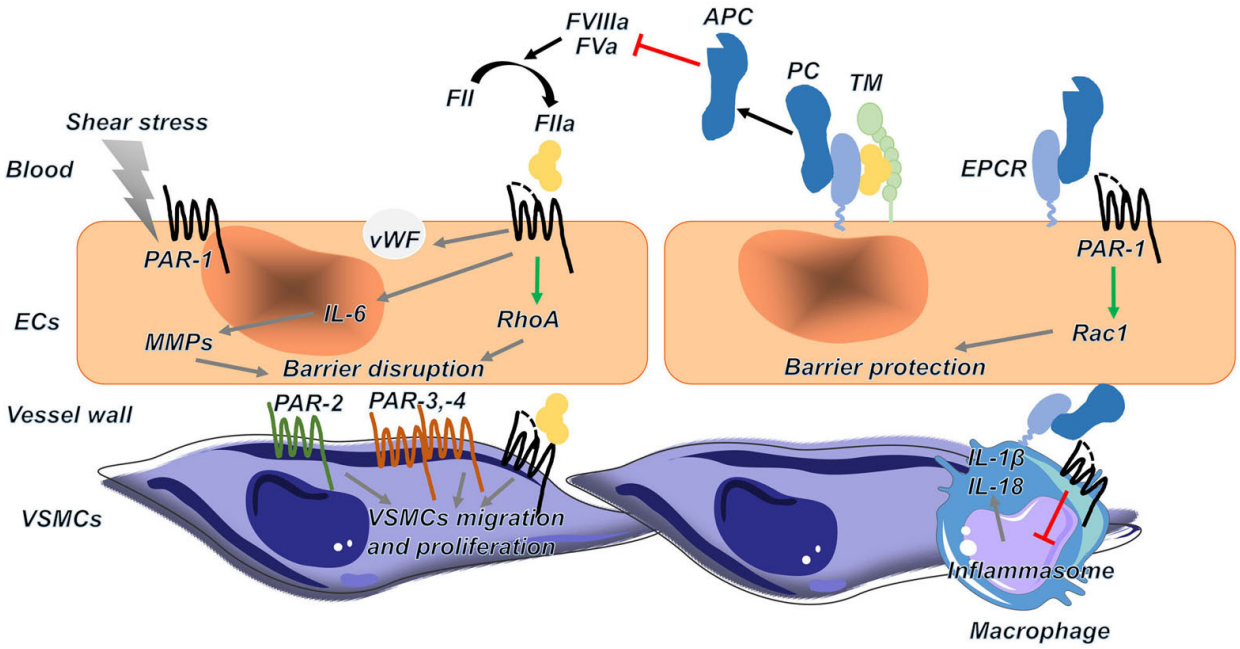

Figure 3. Cellular action of coagulation factors mediated through protease activated receptors. Shear stress stimulates synthesis of protease activated receptor-1 (PAR-1) by endothelial cells (ECs). Activated factors VIII and V (FVIIIa, FVa) promote the generation of thrombin (FIIa) and the activation of PAR-1 by thrombin stimulates von Willebrand factor (VWF) release by ECs. PAR-1-dependent production of interleukin-6 (IL-6) and metalloproteinases (MMPs) and RhoA signaling lead to the disruption of the EC barrier. In addition, FIIa can activate PAR-2 and PAR-4 with its cofactor PAR-3 and stimulates vascular smooth muscle cells (VSMCs) migration and proliferation. The interaction of FIIa with thrombomodulin (TM) and the endothelial protein C receptor (EPCR) leads to activation of protein C (APC) which can also activate PAR-1 and though Rac-1 and the monocyte inflammasome inhibition results in vascular protection. Black curved arrow: "conversion to"; green arrows: "activation of"; red T-bar: "inhibition of"; grey arrows: additional pleiotropic effects.

like a cofactor of PAR-4 in the presence of thrombin activation (Figure 3) (79). The ability of PARs to form heterodimers consisting of different PAR isoforms makes it very challenging to disentangle the signaling properties of PARs in health and disease.

Noncanonical activation of PARs is also reported. Some proteases can cleave the receptors and trigger TL-dependent activation of the receptor. This activation can occur with APC on PAR-1 (see APC section) (80). Triggered signaling pathways differ depending on the way of activation of these receptors. G-protein or β-arrestin activation are the most common activated pathways triggered by PARs (81).These receptors via their role in agonist release, kinase pathway activation can lead to activation of other cellular receptors and even modulate toll-like receptors as well as ion channels (77).

Regarding the actions of PARs in the cardiovascular system, PAR-4 in cardiomyocytes was found to be able to transactivate the epidermal growth factor (EGFR) and ErbB-2 by activation of Src tyrosine kinase, p42/p44 and p38 MAPK (82). In human umbilical vein ECs obtained from preeclamptic pregnancies, PAR-2 mediated expression and release of soluble vascular endothelial growth factor (VEGF) receptor-1 which is also elevated in preeclampsia (83). Bradykinin receptor B2 (B2R) elicits bradykinin-dependent vasodilation, vascular permeability and edema and can interact with PAR-4 (84). Renin angiotensin aldosterone system (RAAS) which also modulates the bradykinin pathway is also related to PARs. Angiotensin II (AngII) upregulate PAR-1 expression via angiotensin-1 receptor in VSMCs (85). Moreover PAR-1 was found to be a strong actor of cardiovascular remodeling in AngII-induced vascular inflammation while FXa and thrombin inhibitors were able to limit the development of experimental aortic aneurysm and atherosclerosis (65,86).

3.3. The role of thrombin on cardiovascular function and diseases

Thrombin is a central coagulation factor leading to polymerization of fibrin fibers and clot formation. Thrombin possesses cellular actions through PARs dependent on hemostasis, like platelet activation and other cellular actions independent of its role in coagulation (Figure 3). Thrombin can activate PAR-1 and PAR-4 and was recently found to be able to activate PAR-2 (87). In the vascular bed, PAR-1 is present at the surface of ECs as well as VSMCs (88–90). In pulmonary arteries thrombin can mediate endothelium-dependent relaxation following thrombin stimulation or trypsin (as a noncanonical activation

of PAR-1) and in endothelium denudated vessels thrombin can mediate vasoconstriction (88).

Thrombin is known to have pleiotropic effects: it can affect differentiation, migration, inflammatory response and gene expression (89,91–94). As for TF, PAR-1 deficiency leads to elevated embryos lethality. PAR-1 knockout embryos died from bleeding suggesting, since PAR-1 is expressed by ECs, that this receptor is implicated in blood vessel development (95).

In pathological conditions, thrombin can act like a growth factor and regulates vascular remodeling through VSMCs proliferation (Figure 2) (96). Vascular remodeling occurs in atherosclerosis, hypertension or restenosis. PAR-1, PAR-2 and PAR-4 mediate VSMCs migration, proliferation and hypertrophy (96,97). In response to shear stress, PAR-1 expression by VSMCs increases and potentiates thrombin-induced proliferation (98). Thrombin is also important in inflammation since it can induce MCP-1 (CCL2) and IL-6 production, or matrix metalloproteinases (MMPs) which will participate in the extracellular matrix degradation and promote vascular remodeling (99–101). Through MCP-1 stimulation, thrombin increases monocyte chemotaxis and participates to immune response modulation in acute or chronic inflammation (102–104). In patients with ascending aortic aneurysm, *in vivo* thrombin generation is increased and correlated to aortic dilatation (105,106). In human atherosclerotic vessel thrombin was found in a concentration sufficient to activate PAR-1 (107). Since thrombin was found within the tunica media, one can postulate that increased thrombin generation participates in vascular wall destabilization, which in turn increases the thrombogenic potential of the vascular cells.

3.4. The direct and indirect effects of APC on vascular cells

PC is a vitamin K-dependent zymogen synthetized in the liver. The single-chain precursor can be cleaved by thrombin and form APC consisting of one light chain and one heavy chain connected by a disulfide bond. APC also possesses a Gla domain able to bind to negatively charged phospholipids and also to the EPCR (108). Conversion of PC to APC is fastened by TM and EPCR. Concerning anticoagulation, APC main function is to inactivate FVa and FVIIIa (109,110). The anticoagulation function can occur when APC is released from EPCR. When it stays linked to this receptor, APC triggers cytoprotective effects. Many beneficial cellular effects of APC were described, from endothelial barrier protection to limitation of tumor proliferation (111). In stroke and ischemia, APC was found to be increased (112,113). Circulating levels of APC are also inversely associated with stroke (114).

APC-dependent improvement of the endothelial barrier was found to be beneficial in sepsis where complications such as hypotension, swelling or inflammation occur (115). Indeed, the effect of APC was well studied in sepsis since the achievement of the recombinant human protein C world-wide evaluation in severe sepsis (PROWESS) trial in the early 2000s (116). Endothelial dysfunction is one of the characteristics of sepsis and can be reduced by APC, but in 2011 the PROWESS-SHOCK trial did not reveal an improved survival in septic shock patients treated with drotrecogin, a recombinant APC (117,118).

During recent years, the interest for the crosstalk between APC, immune cells and vascular function has steadily grown (119). The cytoprotective effect of APC is present in monocytes, macrophages and neutrophils. In myocardial infarction, ischemia-reperfusion injury induces release of proinflammatory cytokines like IL-1β and IL-18 controlled by Nlrp3 inflammasome (120). Nlrp3 expression leads to the oligomeric inflammasome complex formation and IL1β and IL-18 maturation (121). Nazir *et al* found that APC protects from ischemia-reperfusion injury by inhibition of the inflammasome activation in macrophages, cardiomyocytes and cardiac fibroblasts via PAR-1 signaling and mammalian target of rapamycin complex 1 (122). These effects occur through activation of PAR-1 when APC is bound to EPCR (Figure 3). Anti-inflammatory effects on ECs and leukocytes are well described. APC-dependent PAR-1 signaling includes Beta-arrestin-2, PI(3)K/Akt and Rac1 (102,123). PAR-3 can also be involved in APC-dependent cytoprotection (124). The effect of APC can also be mediated by β1 and 3 integrins, apolipoprotein E receptor, macrophage antigen-1 (MAC-1) and Tie2 (125–128). Recent findings showed that in sepsis and stroke PAR-1 biased signaling through cleavage in the R46 position (while thrombin and APC can cleave the R41 of PAR-1) is responsible for the beneficial effect of APC (129).

Soluble EPCR was discovered to bind activated neutrophils through proteinase-3 as well as monocytes with MAC-1, helping them to cross the endothelial barrier (125,130). Considering that circulating EPCR is increased with hypertension, these results suggest a direct link between EPCR and the propagation of vascular inflammation (44).

3.5. From FXII to FXI and bradykinin

Coagulation FXII plays a pivotal role in the crosstalk between hemostasis, immunity and vascular function (131). FXIIa activates FXIa leading to thrombin generation and fibrin clot formation but also has an important role in inflammatory response since it can generate bradykinin (BK) through kallikrein cleavage of HK (Figure 4) (131).

FXII is a 80 kDa single chain polypeptide zymogen. It contains a fibronectin type II and I domain,

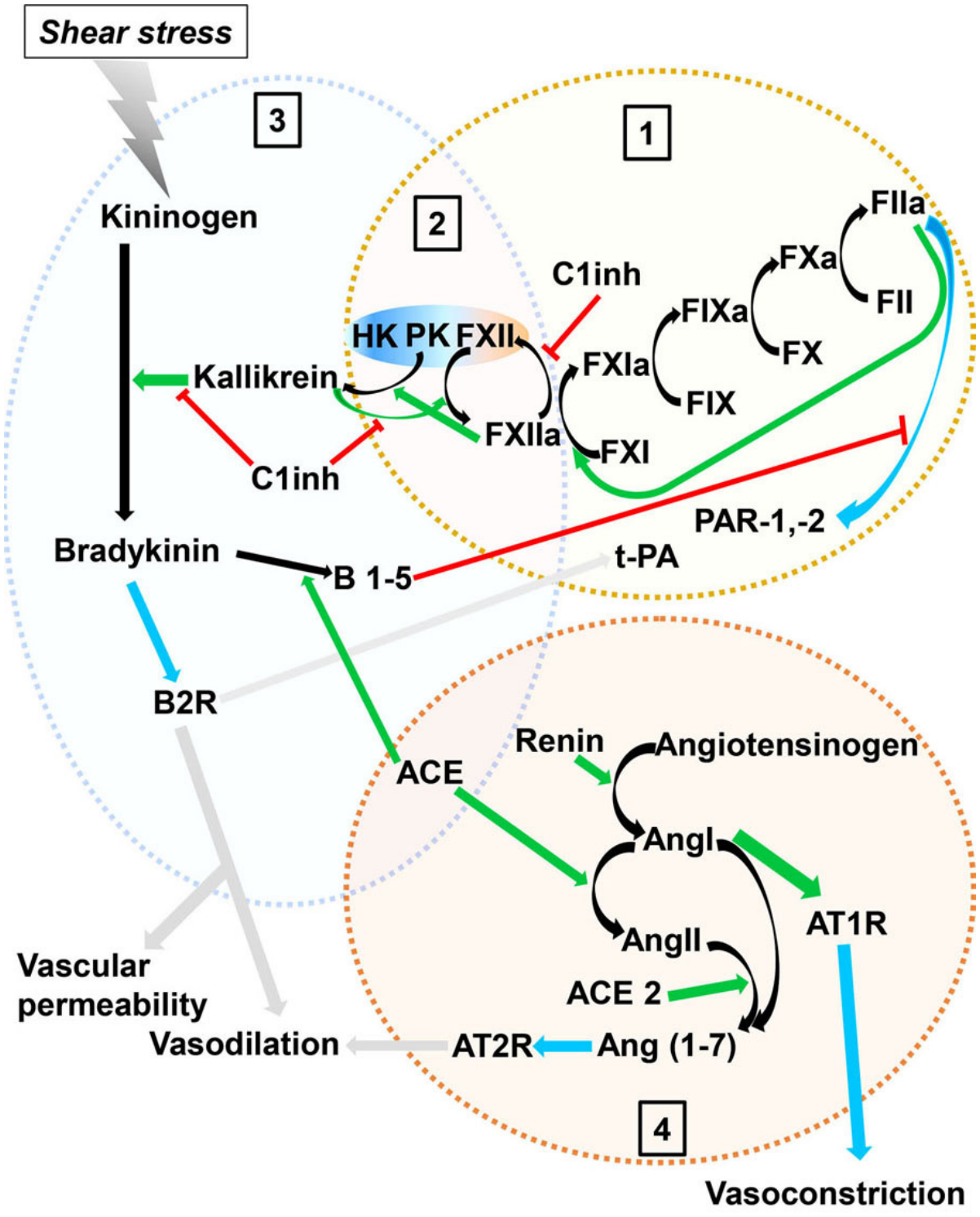

Figure 4. Interplays between coagulation, the kinin-kallikein and the renin-angiotensin systems. 1: In the intrinsic pathway of coagulation activated factor XII (FXIIa) activates factor XI (XIa), which then will activate factor IX (FXIa), followed by factor X activation (FXa) and conversion of prothrombin (FII) to thrombin (FIIa). FIIa amplifies its own activation through FXI activation. 2: High weight kininogen carries prekallikrein (PK) and Factor XII (FXII). Activated FXII (FXIIa) activates PK to kallikrein which in return can activate FXII. 3: Shear stress increases the kininogen syntesis, which by kallikrein can be converted to bradykinin. Bradykinin can bind to its second receptor (B2R) and trigger vascular permeability and vasodilation. The C1 esterase inhibitor (C1inh) can inhibit both FXII and PK activation. 4: Renin converts angiotensinogen to angiotensin I (AngI) and angiotensin converting enzyme (ACE) converts AngI into angiotensin II (AngII). ACE can also degrade bradykinin into bradykinin 1-5 (B 1-5), which is able to inhibit the thrombin-dependent activation of PARs. AngII acts on angiotensin receptor 1 (AT1R) for vasoconstriction while its degradation product angiotensin (1-7) (Ang (1-7)) acts on angiotensin receptor 2 (AT2R) to trigger vasodilation. Black arrows: “conversion to”; green arrows: “activation of”; blue arrows: receptor activation; red T-bar: “inhibition of”; grey arrows: additional pleiotropic effects.

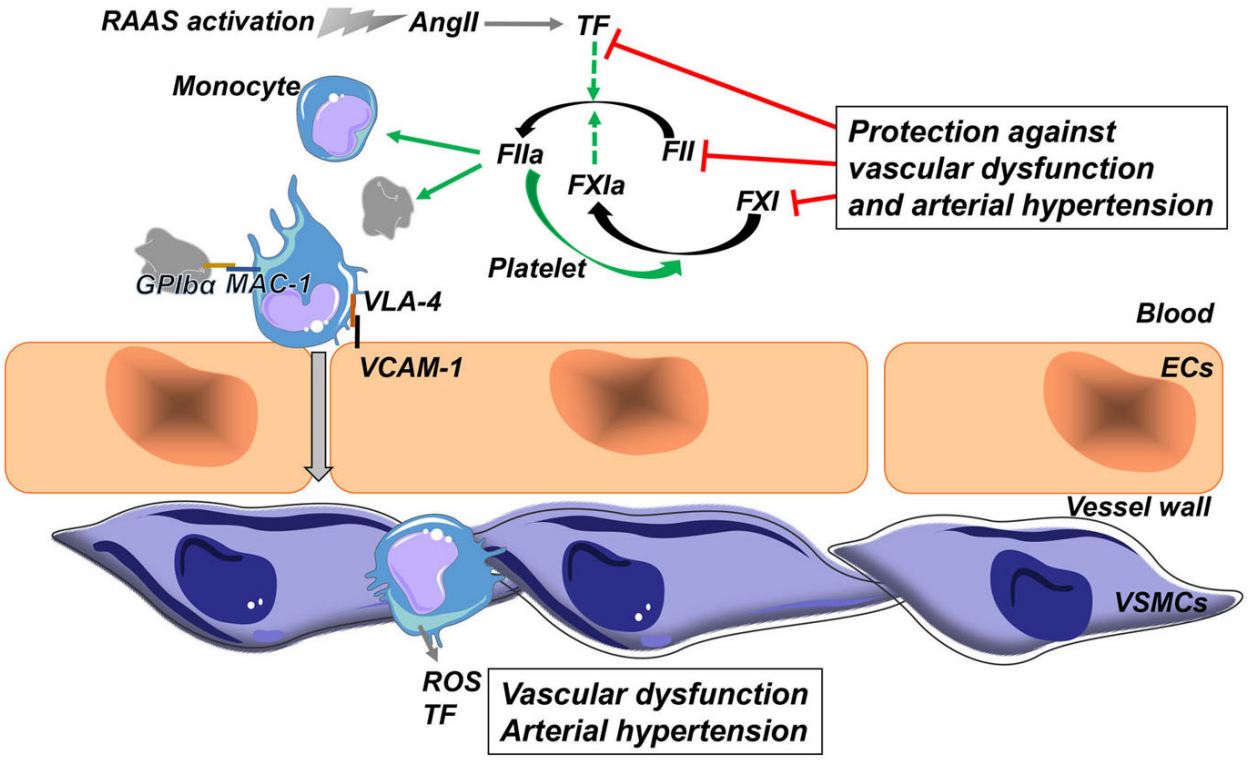

Figure 5. The FXI-related vascular coagulation proinflammatory circuit in hypertension developpement. Overactivation of the renin angiotensin aldosterone system (RAAS) increases angiotensin II (AngII) production, which exerts proinflammatory effects leading to increased tissue factor synthesis (TF). Increased stimulation of the extrinsic pathway and amplification of prothrombin (FII) conversion to thrombin (FIIa) via the Factor XI (FXI) amplification loop at the platelet surface leads to platelet and monocytes activation. The binding of immune cells to the vascular endothelium through membrane receptors assists the transmigration and promotes adverse effect of immune cells whithin the vascular wall and the development of vascular dysfunction. Inhibition of FIIa generation or blockade of the amplication loop via FXI inhibition limit the development of vascular dysfunction. Black arrows: "conversion to"; green arrows: "activation of"; red T-bar: "inhibition of"; grey arrows: other effects. GP: Glycoprotein Ibα; MAC-1: Macrophage antigen-1; VLA-4: Very Late Antigen-4 also known as Integrinα4β1; VCAM-1: Vascular cell adhesion protein 1. ROS: reactive oxygen species; ECs: endothelial cells, VSMCs: Vascular smooth muscle cells.

2 EGF-like domains, a kringle domain and a proline-rich region (132,133). Beyond its function in the coagulation cascade, FXII can act as a growth factor and promotes angiogenesis and cell proliferation (134,135). FXII can bind to the ECs via a multiprotein receptor complex constituted of urokinase plasminogen activator receptor (uPAR), gC1qR and cytokeratin (136). FXII was found to be present and active in atherosclerotic plaques38. Proliferation and angiogenesis action of FXII are mediated by uPAR, integrin β1 and EGFR.

The main direct effect of FXII in vascular function comes from its ability to activate the kallikrein-kinin pathway. Kallikrein activation mediated by FXIIa leads to the cleavage of HK and the release of BK that will induce hypotension and increase vascular permeability through BK receptor 2 (B2R) (137). B2R is a G-protein coupled receptor expressed constitutively and ubiquitously. This pathway plays a major role in hereditary angioedema, a life-threatening tissue swelling disorder. Deficiency of C1-esterase inhibitor (C1INH) which inhibits FXIIa in normal condition leads to excessive BK production and vascular permeability. C1INH deficiency or activity alteration are the cause of hereditary angioedema type I and II (138). One type of hereditary angioedema occurring with normal C1INH is associated with a mutation of FXII in which two missense mutations were detected (139). This type of hereditary angioedema is predominant in women, is hormone-dependent and can be treated with B2R inhibitor, C1INH concentrate or tranexamic acid as for Type I and II hereditary angioedema but also with progestin (140).

FXI, through its ability to be activated by thrombin and to increase thrombin generation, may play a direct role in hypertension and vascular dysfunction development. Increased angII production promotes TF synthesis and release and thrombin generation. Inhibition of FXI in angII infused mice and rats limit blood pressure increase and adverse effect of hypertension (Figure 5) (104). In this pathological setting FXI shows a dichotomy with FXII since beneficial effects of FXI inhibition were lost when only FXII-dependent activation of FXI was inhibited. Moreover contrary to FXI deficient mice, FXII deficient mice were not protected against angII-induced vascular dysfunction. The contrast between FXI and FXII in

thrombus propagation is already well established and thrombin-dependent activation of FXI is one of the main contributors to thrombus propagation (141).

Limiting thrombin generation via FXI inhibition reduces the recruitment of proinflammatory monocytes to the vessel wall and subsequent vascular inflammation development. In patients with acute coronary syndrome FXIa and thrombin generation where found to be elevated (142). FXI deficiency in apolipoprotein E knock-out mice protected against atherogenesis (143). Downstream FXI activation and the intrinsic pathway, FVIII was found to be produced by pulmonary EC and its expression may modulate pulmonary thrombosis and hypertension (144).

3.6. Fibrinogen and clot-related molecules

Fibrinogen is often seen as a biomarker of inflammation and thrombotic risk. It is well described in chronic inflammatory diseases affecting the arterial wall, like hypertension, atherosclerosis or coronary artery disease (145–147). Fibrinogen formation is upregulated by pro-inflammatory cytokines like IL-6 and can also trigger IL-6 and other proinflammatory molecules, such as TNFα or IL-1β (148,149). Fibrinogen as well as fibrin and fibrin degradation products all possess proinflammatory properties and can alter the VSMCs phenotype (148,150). Permeability of the endothelium as well as ECs migration were also increased following fibrin degradation product stimulation (151). In spontaneously hypertensive and hyperlipidemic rats, the plasmatic concentration of fibrinogen was found to be increased (28). On the contrary, cytokines conveying protection from vascular alteration involved in atherosclerosis development, such as IL-4, IL-10 and IL-13, downregulate fibrinogen synthesis (152). Altogether, these data suggest that fibrinogen is involved in progression of vascular disease and directly in their development.

FXIIIa crosslinks and stabilizes the fibrin-clot and can influence VSMCs migration (153). Its plasmatic concentration may not be associated with blood pressure, but it might be associated with atherosclerosis prediction in systemic lupus erythematosus (154). Immune cells, in particular monocytes, are known to participate in atherosclerosis or hypertension progression and FXIII is able to cross-link the angiotensin receptor-1 on monocytes, allowing a full activation of these cells by angII (155,156). In the pathological setting of myocardial infarction, the lack of FXIII in mice led to impaired wound healing with imbalanced extracellular matrix turnover due to overexpression of MMP-9 (157).

Fibrinolysis can occur when t-PA converts plasminogen to plasmin. B2R and Beta 2 AR are known to form heterodimers and bradykinin as well as adrenergic receptors can upregulate t-PA release while beta blockade abolished this t-PA release (158,159). U-PA can also modulate migration of VSCMs submitted to pulse pressure while the increase of PAI-1 inhibits these effects (160,161).

4. CONCLUSION

Coagulation factors are not only important for hemostasis. Their role in vascular function regulation are now starting to be revealed. Through cellular receptors (e.g. PARs, EPCR and TF), FX, thrombin or APC can all exert cellular effects on blood cells and cells that constitute the vascular wall. An number of important questions remains concerning the ability of many cell types to synthetize coagulation factors that could have localized cellular effects. More work is also necessary to understand how circulating coagulation factors could move from the blood to the vascular wall (and inversely) and how this movement is modulated under pathological conditions as well as how cellular effects of coagulation factors could play a role in the vascular regulation of these pathologies.

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JL and PW wrote the manuscript.The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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