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REVIEW ARTICLE

Specificity of coagulation factor signaling

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Summary. Coagulation serine proteases signal through protease-activated receptors (PARs). Thrombin-dependent PAR signaling on platelets is essential for the hemostatic response and vascular thrombosis, but regulation of inflammation by PAR signaling is now recognized as an important aspect of the pro- and anti-coagulant pathways. In tissue factor (TF)-dependent initiation of coagulation, factor (F) Xa is the PAR-1 or PAR-2-activating protease when associated with the transient TF–FVIIa–FXa complex. In the anticoagulant protein C (PC) pathway, the thrombin–thrombomodulin complex activates PC bound to the endothelial cell PC receptor (EPCR), which functions as a required coreceptor for activated PC-mediated signaling through endothelial cell PAR-1. Thus, the pro- and anti-inflammatory receptor cascades are mechanistically coupled to immediate cell signaling, which precedes systemic coagulant or anticoagulant effects. In contrast to the substrate-like recognition of PARs by thrombin, TF- or EPCR-targeted activation of PARs generates cell-type specificity, PAR selectivity and protease receptor cosignaling with the G-protein-coupled PAR response. Protease receptors are thus major determinants of the biological outcome of coagulation factor signaling on vascular cells.

Keywords: coagulation, protease-activated receptors, signaling, tissue factor.

Introduction

In vertebrates, the activation of the coagulation cascade by tissue factor (TF) is central to both the repair of tissue injury and the host defense towards microbial pathogens. Cells of the innate and acquired immune system participate in the interdependent pathways of coagulation and inflammation. Activation of coagulation promotes inflammation, evidenced by the inflammatory component in clinical aseptic deep vein thrombosis and by a variety of animal models that show inflammatory effects on the coagulation cascade [1–4]. In septicemia, the TF-initiated coagulation pathway drives an inflammatory escalation that is independent of fibrin formation and microthrombosis, strongly indicating important pro-inflammatory cell signaling events of coagulation serine proteases [5–9]. Conversely, the protein C (PC) pathway counteracts systemic inflammation and activated PC (APC) has now been approved as therapy to reduce the mortality in severe sepsis [10–12]. This review deals with current concepts of how the initiation of the pro- and anti-coagulant pathways is mechanistically linked to signaling through protease-activated receptors (PARs). The paradigm is emerging that the biological effects of PAR activation are highly dependent on protease-binding coreceptors that determine specificity of coagulation factor signaling on vascular cells.

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Thrombin-mediated PAR signaling

Platelet deposition is an integral part of the normal hemostatic response, and significantly contributes to intravascular thrombosis. Thrombin-dependent platelet activation has served as the paradigm for signaling by coagulation serine proteases [13]. Thrombin cleaves an extracellular peptide bond in PAR-1, followed by binding of the tethered neo-aminoterminus to the ligand-binding pocket of the G-protein-coupled receptor. PAR-1 cleavage by thrombin follows the general rules of serine protease substrate recognition that involves binding of the scissile bond to the catalytic cleft as well as interactions with adjacent extended substrate binding sites (exosites) that increase substrate–enzyme affinity. The acidic, hirudin-like sequences of PAR-1 or PAR-3 interact with thrombin's basic exosite I, and substrate-like binding of thrombin to the PAR is crucial for receptor activation (Fig. 1A). Occupancy of exosite I by thrombomodulin severely impairs thrombin's ability to activate PAR-1 [14–16]. PAR-4, which lacks the hirudin-like recognition sequence for exosite I, is a fairly poor substrate for thrombin [17]. Both findings highlight the importance of exosite I binding for signaling of the fluid phase enzyme, thrombin.

The prototypic model that thrombin signals by binding to a PAR followed by activation of the same receptor was challenged by the finding that either PAR-1 or PAR-3 is required to localize low concentrations of thrombin on the platelet surface for efficient cleavage of PAR-4 [17–19]. G-protein-coupled receptors can form heterodimers, and heterodimerization between PAR-1 and PAR-4 (human platelets) or PAR-3 and PAR-4 (murine platelets) may provide the proximity that is required for

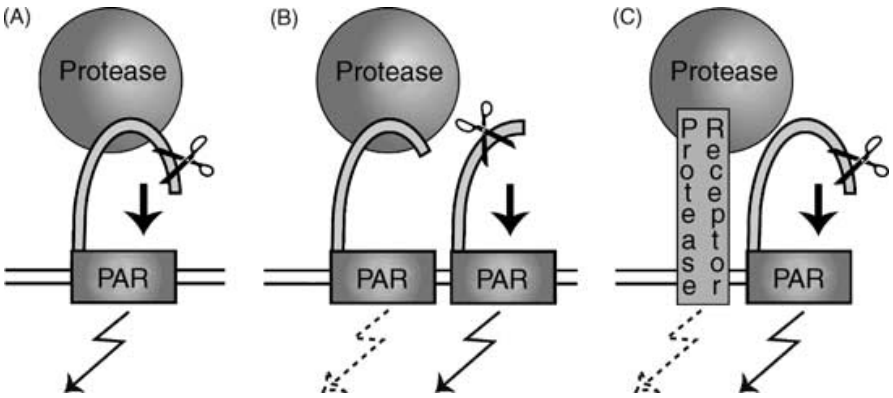

Fig. 1. Schematic models for proteolytic PAR activation. (A) Prototypic model of PAR-1 cleavage by thrombin. (B) Coreceptor model in which PAR-1/3 serves as a thrombin binding receptor that enhances PAR-4 cleavage. PAR-1 cleavage can produce cosignaling with PAR-4 in human platelets. (C) Model for coreceptor-dependent PAR activation of coagulation protease receptors. Although these receptors are not signal transducers, they may nevertheless confer PAR specificity and cosignaling by intracellular recruitment of adaptor molecules.

the observed receptor crosstalk (Fig. 1B). Murine PAR-3 does not signal directly, and PAR-3 thus solely serves as a coreceptor that increases the efficiency of PAR-4 cleavage by thrombin [19]. In contrast, human PAR-1 signals independently, and the response of human platelets to thrombin is a composite of the more rapid and transient response of PAR-1, followed by more sustained PAR-4 signaling [20,21]. Thrombin signaling in endothelial cells illustrates a variation of the theme of cooperation between PARs. When direct PAR-1 signaling is blocked with a small molecule antagonist, thrombin-cleaved PAR-1 can activate PAR-2 [22]. This unidirectional cross-activation is explained by the ability of the neo-aminoterminus of PAR-1 to function as an agonist for PAR-2, but not vice versa [23]. Because most cell types express more than one PAR, receptor crosstalk between PARs accounts in part for the combinatorial diversity of the PAR response *in vivo*.

PAR activation by other proteases

PAR-2 is not cleaved by thrombin, but is activated by the fairly non-selective protease trypsin [24]. The activation region of PAR-2 shows no distinct features of protease specificity, leading to the concept of a 'generic PAR' that serves as the signal transducer for a variety of proteases. It is well appreciated that for the coagulation cofactor complexes, a perfect fit of scissile bond and the catalytic cleft of proteases is not required for cleavage of substrates that are appropriately docked with the activator complexes [25,26]. Proper positioning of the scissile bond of PAR-2 relative to a membrane-associated protease can similarly allow for biologically relevant signaling by any of the identified PAR-2 cleaving proteases, which include mast cell tryptase [27], membrane-type serine protease 1 [28], sperm protease acrosin [29], bacterial proteases [30] and coagulation factors (F)VIIa and FXa [31,32]. The lack of PAR-2 cleavage by thrombin shows that the scissile bond may prevent cleavage by a specific protease. However, the unique thrombin-complementary extracellular domain of PAR-1 does not restrict activation by other proteases. PAR-1 can be activated by FXa [33,34], plasmin [35] or activated protein C (APC) [36], as well as by pathogen-derived, arginyl-specific cysteine proteases (gingipains) [37]. With suboptimal scissile bond complementarity of PARs and the catalytic clefts, coreceptors are crucial to target proteases to PARs and to increase efficiency of PAR cleavage (Fig. 1C).

Coupling of PAR signaling to the initiation of coagulation by TF

In the cellular initiation of coagulation, TF binds FVIIa to render FVIIa catalytically active. The TF–FVIIa complex then binds FX through multiple extended substrate docking sites, followed by conversion of FX to FXa [26,38]. FXa can cleave PAR-1 and PAR-2, but cell signaling has been reported at FXa concentrations that were higher than those expected to occur physiologically [31,33,34,39]. A unique feature of the TF initiation reaction is the transient stability of the TF–FVIIa–FXa product complex that exists prior to the release of product FXa. The TF–FVIIa–FXa complex (see model in Fig. 2A) can be trapped with a highly specific nematode-derived inhibitor, NAPc2, which stabilizes the ternary complex by inhibiting FVIIa, while maintaining FXa in a catalytically active conformation [40]. The NAPc2-stabilized ternary complex efficiently signals through PAR-1 and PAR-2 in heterologous expression systems, and predominantly through PAR-2 on cytokine-stimulated endothelial cells [32]. Quantitative analysis of the dose–response of cell signaling induced by free FXa, vs. FXa that is transiently associated with TF–FVIIa in the initiation complex, demonstrates at least 5-fold enhanced cell signaling of the ternary TF–FVIIa–FXa complex [32]. Because FXa exerts its procoagulant activity only after dissociation from TF–FVIIa, cell signaling by the TF–FVIIa–FXa complex precedes the coagulant effects of the TF-dependent coagulation pathways. Cell signaling is thus directly coupled to the mechanism of TF-dependent initiation of coagulation.

Work from several laboratories showed that the binary TF–FVIIa complex can also activate typical G-protein-coupled receptor pathways in fibroblasts and epithelial cells [41–51]. We found no efficient signaling of TF–FVIIa under conditions where the ternary complex potently activated endothelial cells [32]. Figure 2(B) shows an experiment with the human keratinocyte cell line HaCaT, a model system for TF–FVIIa-dependent signaling [47,49,50]. We used the induction of the MAP kinase pathway-dependent nuclear orphan receptor TR3 as a quantitative readout for PAR activation. Even at the supraphy-

siological concentration of 50 nmol L^{-1} FVIIa, TR3 induction was essentially undetectable, which is contrasted by potent gene induction by the NAPc2-stabilized TF–FVIIa–FXa complex or by initiation of coagulation at near-plasma concentrations of FVIIa and X. Although a novel PAR has been discussed as the transducer of TF-dependent signaling in baby hamster kidney cells [52], HaCaT cells express PAR-2, and signaling by both the ternary complex and free FXa was efficiently inhibited by inhibitory antibodies to PAR-2. These data show that the ternary complex signaling mechanism functions on epithelial cells, and suggest that the known PARs are generally the relevant targets for cofactor-presented coagulation proteases.

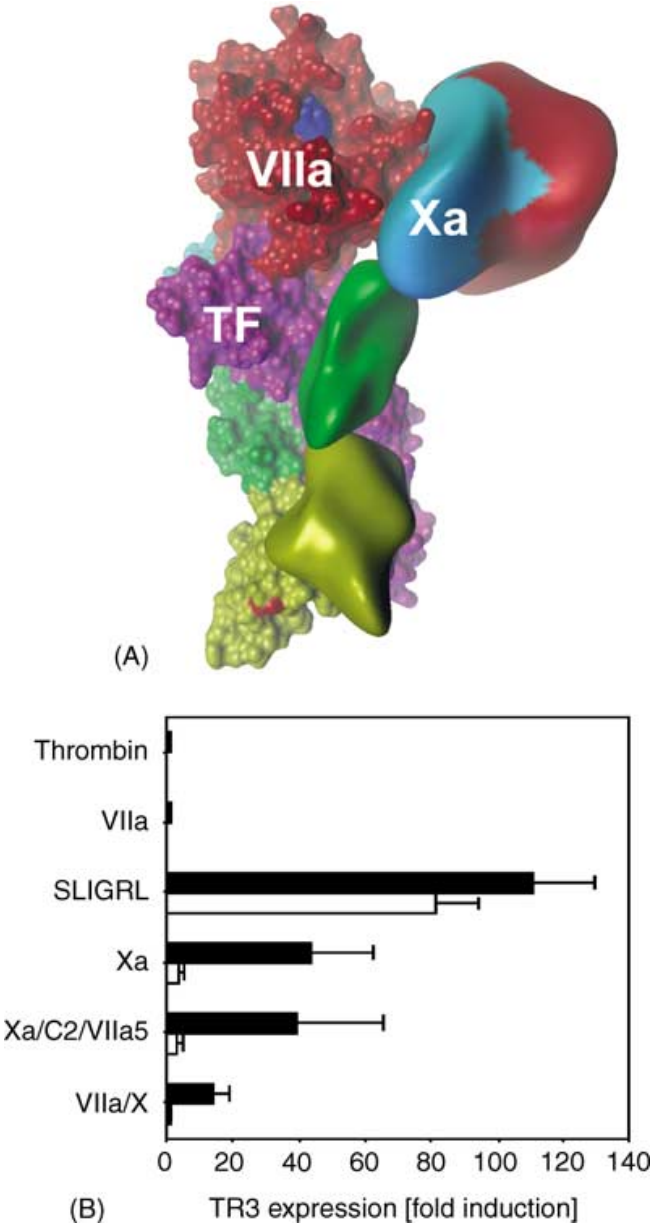

Fig. 2. Signaling by the TF–FVIIa–FXa complex. (A) Molecular model of the TF-FVIIa-FXa complex showing the extended recognition surface by which FXa can transiently stay associated with TF–FVIIa. (B) PAR-2-dependent gene induction in HaCaT cells by the ternary coagulation initiation complex. Induction of TR3 in serum-starved confluent HaCaT keratinocytes by 5 nmol L^{-1} thrombin, 50 nmol L^{-1} FVIIa, 100 mol L^{-1} PAR-2 agonist peptide SLIGRL, 50 nmol L^{-1} FXa, 20 nmol L^{-1} FXa/100 nmol L^{-1} NAPc2/10 nmol L^{-1} active site mutated FVIIa (FVIIa5), or 5 nmol L^{-1} FVIIa/100 nmol L^{-1} zymogen FX (X). Preincubation for 15 min with 300 μg mL^{-1} polyclonal anti-PAR-2 IgG (open bars) demonstrates signaling of FXa through PAR-2.

Signaling of the anticoagulant pathway on endothelial cells

In the anticoagulant PC pathway, thrombomodulin binds thrombin and changes its specificity from procoagulant functions to PC activation [53]. In addition, the endothelial cell PC receptor (EPCR), an endothelial cell-specific receptor for both PC and APC, plays a critical role by enhancing the activation of PC by the thrombin–thrombomodulin complex [54]. Fibroblasts from PAR-1-deficient mice express neither EPCR nor any of the known PARs. Coexpression of PAR-1 or PAR-2 with EPCR renders the cells responsive to proteolytic signaling by low, physiologically achievable concentrations of APC ($<$10 nmol L^{-1}). An APC-binding-deficient mutant of EPCR does not support APC-dependent PAR signaling, confirming that EPCR serves as a binding receptor that helps to orient APC for activation of PARs [36]. Figure 3 shows APC signaling in melanoma cells that express PAR-1, -2 and -3 constitutively rather than by overexpression, as in the fibroblast model. These cells also require transfection with EPCR to become APC-responsive. APC signaling is dependent on proteolysis, as shown by the complete inhibition by α1 antitrypsin Pittsburg, which is a potent inhibitor of thrombin and APC [55]. However, APC signaling is not indirectly caused through other proteases, e.g. FXa inhibitors do not block APC signaling (Fig. 3), and we

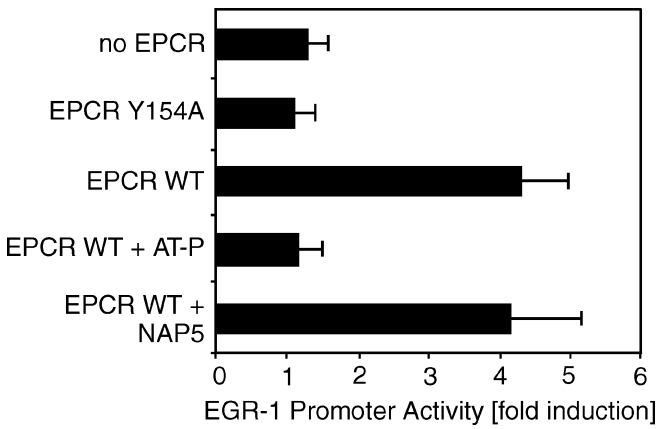

Fig. 3. Proteolytic APC signaling is dependent on EPCR. APC (10 nmol L^{-1}) induces signaling (measured by EGR-1 promoter activity induction) in M2 melanoma cells expressing wild-type EPCR, but not the Y154A EPCR mutant deficient in APC binding. Signaling is blocked by the APC inhibitor α1-antitrypsin Pittsburgh (AT-P, 1 μmol L^{-1}), but not by the FXa inhibitor NAP5 (1 μmol L^{-1}). Hirudin (100 nmol L^{-1}) is present throughout.

use hirudin routinely in all our experiments to block potential thrombin signaling. The APC–EPCR complex thus signals by proteolytically activating PARs.

Human umbilical vein endothelial cells (HUVECs) express PAR-1, PAR-2, and EPCR. Unexpectedly, cleavage-blocking antibodies to PAR-1 completely prevents APC-mediated MAP kinase phosphorylation, indicating that APC signaling in endothelial cells is entirely PAR-1-dependent [36]. The signaling of APC in endothelial cells has been analyzed further by microarray gene-expression profiling. All genes that were upregulated by APC signaling were also induced by PAR-1 agonist peptides [36]. These data demonstrate on a large scale the similarity of PAR-1 and APC signaling. The crucial role of PAR-1 in APC signaling was further confirmed by antibody-blocking experiments that clearly showed that APC-mediated upregulation of antiapoptotic and endothelial protective genes is mediated by PAR-1. The PAR-1-mediated APC response is also consistent with previously demonstrated anti-inflammatory and antiapoptotic effects of APC [56]. What determines selectivity for the activation of PAR-1 on endothelial cells is unclear. Signaling by G-protein-coupled receptors can induce metalloproteinase activity [57], and thrombin stimulation releases EPCR from endothelial cells in a metalloproteinase-dependent process [58,59]. PAR-1, but not PAR-2, agonist peptides induce this shedding of EPCR, which may indicate that PAR-1 and EPCR are colocalized in a functional microdomain on endothelial cells.

The identification of PAR-1 as the target for signaling of the anticoagulant PC pathway raises several puzzling questions: (i) How can the thrombin receptor PAR-1 be a relevant receptor for APC signaling, if thrombin triggers the anticoagulant PC pathway on endothelial cells? (ii) Thrombin signaling through PAR-1 is pro-inflammatory in several experimental models. How can APC achieve therapeutic benefits in the escalation of sepsis by activating the same receptor? (iii) If the pro- and anti-coagulant pathways utilize similar signaling receptors on vascular cells, how do these receptor cascades generate specificity of the biological response?

Intravascular thrombin signaling

PAR-3- and PAR-4-deficient mice are similarly protected from intravascular, platelet-dependent thrombosis, demonstrating that PAR-3 is a necessary coreceptor for thrombin signaling on platelets [60,61]. Because murine PAR-3 serves solely to concentrate thrombin locally for efficient PAR-4 cleavage, the similar phenotype of PAR-3 and PAR-4 knockout animals indicates that thrombin is generated intravascularly at a threshold concentration that cannot overcome the decreased thrombin affinity of PAR-3-deficient platelets. Inefficient thrombin-dependent platelet activation is also documented by an elegant study in which thrombin was infused into primates [62]. As expected from the trapping of thrombin by endothelial cell thrombomodulin, low concentrations of thrombin enhanced the generation of APC in the circulation, but markers of platelet activation were not elevated. The inability of low concentrations of circulating thrombin to trigger PAR-dependent platelet activation strongly supports a model in which thrombin has to be present above a threshold concentration to elicit direct intravascular signaling through PARs.

Exosite I interactions are an important regulator of thrombin signaling. Cell surface or soluble thrombomodulin attenuates PAR-1 cleavage [15,16,63]. Fibrinogen also interacts with exosite I and thus acts as a competitive inhibitor of PAR-1 activation, because of its high plasma concentration relative to generated thrombin. Fibrinogen is an acute-phase protein, and increased levels during inflammation may shift the threshold for direct thrombin signaling. Below this threshold, thrombin may predominantly signal indirectly through the PC pathway, targeting PAR-1 specifically on endothelial cells (Fig. 4). In contrast, direct thrombin signaling is not endothelial cell-restricted and can target a variety of extra- and intra-vascular

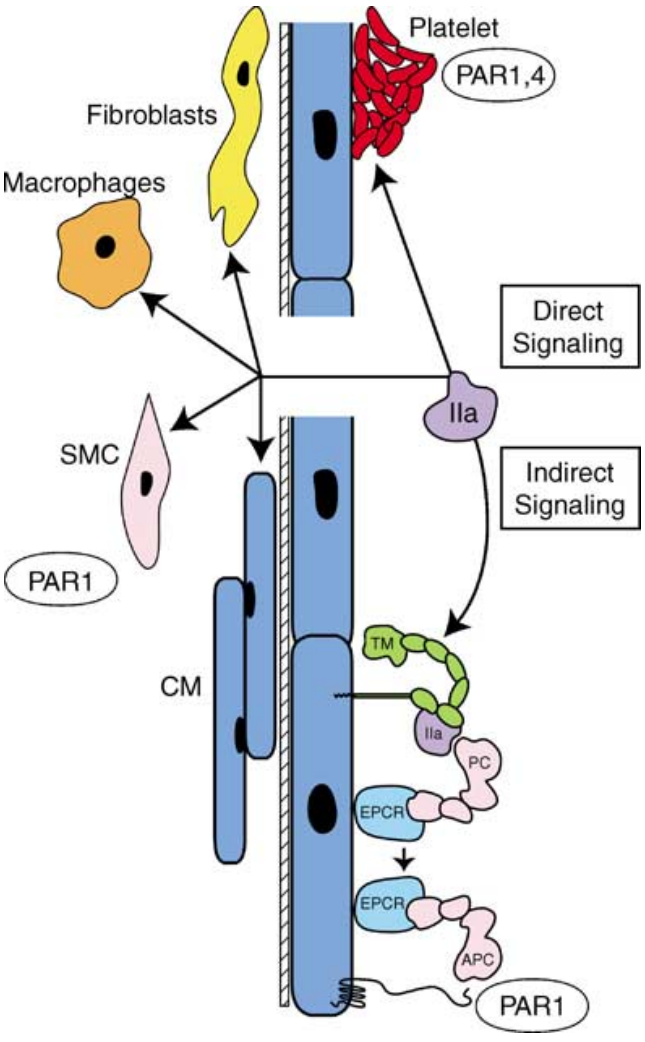

Fig. 4. Thrombin signaling pathways. Schematic overview of direct thrombin signaling that occurs intravascularly at sites of platelet deposition and extravascularly targeting a variety of cell types (SMC, smooth muscle cells; CM, cardiomyocytes). Under physiological conditions, intravascular thrombin predominantly signals indirectly through the EPCR–APC–PAR-1 pathway.

cells. Indeed, experimental models that have documented inflammatory effects of thrombin are characterized by extravascular fibrin deposition, indicating that thrombin signaling occurs in the extravascular space [1–4]. Lower fibrinogen concentration in extravascular exudates, together with the absence of thrombomodulin, favor direct thrombin signaling. Notably, experimental models that document thrombin-dependent vascular effects are frequently carried out in plasma-free buffers or apply thrombin from the extravascular side of the vessel, which eliminates competition by fibrinogen and protease inhibitors in the plasma [14,64]. The extracellular milieu in which thrombin is generated thus significantly impacts on whether thrombin signaling is direct or indirect via the PC pathway, which appears to be preferred intravascularly under non-inflammatory conditions.

Anti-inflammatory signaling of the protein C pathway

Pioneering work by Taylor and Esmon showed that infusion with a low concentration of thrombin could block the lethal inflammatory response to endotoxin [65]. Thrombin infusion leads to PC activation, and direct infusion of APC into baboons lethally challenged with *E. coli* confers protection, identifying APC as the relevant downstream effector of thrombin administration [10]. Potent inhibitors of thrombin formation abolished the coagulation abnormalities in the same sepsis model, but did not attenuate shock and the lethal effects of *E. coli* [8], indicating that APC regulates inflammatory responses independent of its anticoagulant effects. APC similarly reduced nitric oxide-mediated hypotension and pulmonary vascular injury, effects that were not reproduced by other thrombin-directed anticoagulants [66,67], confirming that APC does not act primarily to reduce thrombin levels and its pro-inflammatory effects. Further, APC was superior to more potent anticoagulants, such as FXai or heparin, in reducing ischemia/reperfusion renal injury and renal levels of inflammatory cytokines tumor necrosis factor-α and interleukin-8 [68]. Taken together, the current information clearly indicates that potent anti-inflammatory effects of APC are independent of its anticoagulant action.

The finding that APC generation upon thrombin infusion into primates is highly dependent on EPCR [69] links EPCR-dependent APC signaling through PAR-1 to the protective effects of preventive thrombin administration in sepsis models. That expression of PAR-1 in the endothelium is sufficient to rescue the embryonic lethality of PAR-1-deficiency is also consistent with protective functions of PAR-1 signaling in endothelial cells [70]. The EPCR–APC–PAR-1 signaling pathway can be considered a preventive response of the endothelium to imminent intravascular, inflammatory challenge. APC therapy in sepsis was highly efficient in severely ill patients, but there was only marginal benefit in less severe disease [12]. This may suggest that the physiological endothelial protective pathway provides anti-inflammatory benefit in the early stage of sepsis syndrome, while continuing infection leads to progressive disabling of the PC pathway. Thrombomodulin expression rapidly declines in inflammatory cytokine-stimulated endothelial cells [71], which will diminish activation of EPCR-bound PC. By restoring APC bound to EPCR, therapeutically administered APC can reconstitute the signaling component of the PC pathway in thrombomodulin-depleted endothelial cells that, in the microcirculation, frequently retain EPCR expression [72]. Our recent data show that cytokine-stimulated endothelial cells retain responsiveness to APC in an EPCR- and PAR-1-dependent manner. The EPCR-conferred endothelial cell selectivity of APC signaling is the crucial distinction from thrombin signaling, which becomes increasingly prevalent in escalating sepsis. Although thrombin may exert some of the protective APC effects on the endothelium, overall these benefits are readily offset by pro-inflammatory actions of thrombin on other vascular or extravascular cells. The dependence of the APC pathway on two receptors, thrombomodulin and EPCR, creates a unique endothelial cell restriction of PAR-1 signaling that is not recapitulated by the signaling of other coagulation proteases.

Specificity of PAR signaling in vascular cells

PAR-1 and PAR-2 are found coexpressed in most cell types of the cardiovascular system, including smooth muscle cells, fibroblasts, cardiomyocytes, neutrophils, macrophages, mast cells, and endothelial cells. Cell type-restricted expression of PARs is thus an unlikely mechanism to determine the biological specificity of the vascular response to PAR stimulation. Overall, stimulation with PAR-1 or PAR-2 agonist peptides produces very similar second-messenger responses (Ca mobilization, phosphoinositide hydrolysis), phosphorylation of mitogen-activated kinases, and immediate early transcriptional gene induction events documented by large-scale gene expression profiling [36]. However, the monocyte chemoattractant protein-1 transcript is induced by PAR-1, but not PAR-2 agonist stimulation, providing evidence for PAR selective effects in endothelial cells. In cardiomyocytes, only the PAR-1 agonist peptide induces c-Jun aminoterminal kinase and AKT [73], and opposing effects of PAR-1 and PAR-2 agonists on proliferation are documented in keratinocytes [74].

In vivo models also document distinct roles of PAR-1 vs. PAR-2 within the vasculature. PAR-1 and PAR-2 agonist peptide stimulation similarly produce a nitric oxide-dependent hypotensive response by acting on the endothelium, but only PAR-1 activation produces vessel constriction mediated by smooth muscle cells that show other PAR-2 responses [75–78]. Unlike direct PAR-1 stimulation, PAR-4 agonists and thrombin induce leukocyte rolling on the endothelium, and leukocyte rolling is diminished in PAR-2-deficient mice [64,79]. Events that follow receptor activation may play an important role for the observed specificity of PAR signaling. PARs are inactivated by rapid phosphorylation that leads to the recruitment of the adaptor protein β-arrestin, which uncouples G-protein binding to the activated PAR. PAR-2 is internalized by β-arrestin targeting to clathrin-coated pits [80], whereas PAR-1 is desensitized, but not internalized, through β-arrestin-binding [81,82]. The PAR-2–β-arrestin–clathrin complex pre-

vents the nuclear localization of phosphorylated MAP kinase Erk1/2, which reduces proliferation in favor of cytoskeletal effects of PAR signaling [83]. PAR selective effects are thus documented in several cell types that coexpress PARs, and activation of PAR-1 and PAR-2 does not produce entirely redundant cellular responses.

Activation by proteases provides additional mechanisms to generate specificity of PAR signaling. Glycosylation has been shown to be a mechanism by which PAR-2 cleavage by tryptase is selectively reduced [84,85]. Thrombin-sensitive receptors are expressed ubiquitously, but TF is also expressed in several of these cells, indicating a considerable overlap in cell types that are responsive to procoagulant cell signaling. However, cell signaling by TF and thrombin is unlikely to be redundant, because of rate differences that impact on the cellular effect of G-protein-mediated receptor activation. A protease that is bound to a receptor, such as the TF–FVIIa–FXa complex or EPCR-bound APC, can only activate a limited number of spatially close PARs, whereas the action of thrombin is not restricted to particular cell surface areas, resulting in rapid cleavage of essentially all cell surface-expressed PARs [33]. Thrombin thus generates a response with high magnitude, but limited duration, whereas membrane-targeted proteases generate an extended PAR response. FXa and the TF–FVIIa complex indeed produce prolonged MAP kinase phosphorylation, compared with thrombin signaling [33,46]. The duration of MAP kinase activation by G-protein-coupled receptor signaling is critical for downstream gene induction [86]. Prolonged signaling input may explain why the fairly inefficient cleavage of PAR-1 by plasmin and FXa, or of PAR-2 by TF–FVIIa, induces the pro-angiogenic *Cyr61* gene with similar efficiency to thrombin signaling [33,35,42].

Protease receptor-dependent PAR cleavage targets the PAR response to specific cellular microdomains, whereas thrombin cleaves PARs independent of location. The proteolytic release of EPCR is enhanced by overexpression of caveolin, indicating that EPCR can be targeted to glycosphingolipid-rich microdomains (rafts) and caveolae [58]. TF can also translocate to caveolae upon TF–FVIIa–FXa–TFPI quaternary complex formation with glycosyl-phosphatidylinositol-anchored TFPI [87]. This links signaling by the ternary TF–FVIIa–FXa complex to caveolae/rafts that are enriched in key signaling components of G-protein-coupled receptor signaling, including protein kinase C (PKC) α, src tyrosine kinases, adenylate cyclase, phosphatidylinositol-3-kinase [88], and the epidermal growth factor receptor [57]. Receptor targeting to caveolae and signaling is influenced by post-translational modifications, in particular palmitoylation [89]. Both EPCR and TF have an intracellular free cysteine residue that acts as a palmitoylation site, as shown for TF [90], but it has not been specifically investigated whether palmitoylation of EPCR or TF is important for PAR signaling.

PAR-1 signaling is modulated by thrombomodulin that prolongs the duration of nuclear Erk signaling in endothelial cells [91]. This effect is dependent on thrombin binding to thrombomodulin, suggesting direct intracellular cosignaling of thrombomodulin with PARs. Like thrombomodulin, TF has an extended cytoplasmic domain that plays a role in TF-dependent hematogenous metastasis [92,93]. In this experimental model, it is still unclear whether the TF cytoplasmic domain is important for the function of TF in cell adhesion and migration [94,95], or whether there is intracellular crosstalk with PAR signaling. In heterologous expression studies, the TF cytoplasmic domain is clearly not required for the activation of PARs by TF–FVIIa [44,45,49]. The only evidence for a cooperation of the TF cytoplasmic domain with PAR signaling comes from experiments in monocytes. TF–FVIIa signaling enhances reactive oxygen species (ROS) production, an effect that is lost when the TF cytoplasmic domain is truncated [96]. ROS are produced by NAD(P)H oxidase, which is localized to rafts [88]. In order to provide regulatory or cosignaling functions, the TF cytoplasmic domain may need to be specifically localized to these membrane domains. Our recent observation that PKCα-dependent phosphorylation of Ser258 in the TF cytoplasmic domain is dependent on intact rafts/caveolae would support this notion. Mice that lack the TF cytoplasmic domain escape the embryonic lethality of TF deficiency [97,98] and will provide a useful tool to directly test involvement of intracellular cosignaling by the TF cytoplasmic domain in experimental models of tumor progression and inflammation.

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