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Thrombin and vascular inflammation

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Abstract Vascular endothelium is a key regulator of homeostasis. In physiological conditions it mediates vascular dilatation, prevents platelet adhesion, and inhibits thrombin generation. However, endothelial dysfunction caused by physical injury of the vascular wall, for example during balloon angioplasty, acute or chronic inflammation, such as in atherothrombosis, creates a proinflammatory environment which supports leukocyte transmigration toward inflammatory sites. At the same time, the dysfunction promotes thrombin generation, fibrin deposition, and coagulation. The serine protease thrombin plays a pivotal role in the coagulation cascade. However, thrombin is not only the key effector of coagulation cascade; it also plays a significant role in inflammatory diseases. It shows an array of effects on endothelial cells, vascular smooth muscle cells, monocytes, and platelets, all of which participate in the vascular pathophysiology such as atherothrombosis. Therefore, thrombin can be considered as an important modulatory molecule of vascular homeostasis. This review summarizes the existing evidence on the role of thrombin in vascular inflammation.

Keywords Thrombin · Endothelium · Vascular inflammation · Atherosclerosis

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Abbreviations

AT	Antithrombin
APC	Activating protein C
CCL	Chemokine (C–C motif) ligand
$cPLA_2$	Cytosolic phospholipase A_2
CXCL	Chemokine (C-X-C motif) ligand
cysLT	Cysteinyl leukotrienes
DCs	Dendritic cells
ECs	Endothelial cells
EDHF	Endothelium-derived hyperpolarizing factor
ERK	Extracellular signal regulated kinase
EGFR	Epidermal growth factor receptor
GPIb/IIb/IIIa	Glycoprotein Ib/IIb/IIIa
HLA	Human leukocyte antigen
ICAM-1	Intercellular adhesion molecule-1
IFN-γ	Interferon-γ
IL-1α/β	Interleukin-1α/β
IP-10	Inducible protein-10
LT	Leukotriene
LPS	Lipopolysacharide
MAPK	Mitogen activated protein kinase
M-CSF	Macrophage colony-stimulating factor
NO	Nitric oxide
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease-activated receptors
PDGF	Platelet-derived growth factor receptor
PF4	Platelet factor 4
PGE_2	Prostaglandin E_2
PGI_2	Prostacyclin I_2
PMN	Polymorphonuclear leukocytes
PSGL-1	P-selectin glycoprotein ligand-1
RANTES	Regulated on activation, normal T expressed and secreted
TF	Tissue factor

TGF-β	Transforming growth factor-β
TFPI	Tissue factor pathway inhibitor
TNF-α	Tumor necrosis factor-α
TXA_2	Thromboxane A_2
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells
vWF	von Willebrand factor

Introduction

Endothelium, rather than being an intravascular lining merely preventing coagulation, is absolutely crucial for the maintenance and adaptation of the vascular homeostasis both under physiological and pathophysiological conditions. Two principal and contrasting modes of endothelium behavior have been defined: (i) anti-inflammatory and (ii) proinflammatory [1]. Under physiological conditions, endothelium tends to maintain an anti-inflammatory state [2, 3] by mediating vascular dilatation [4, 5], preventing platelet adhesion and activation [6], and by its inhibition of thrombin generation [7]. In addition, endothelium acts to attenuate adhesion and reduces the consequent transmigration of inflammatory leukocytes [8, 9]. Oxygen radicals generated during normal cell metabolism are efficiently scavenged to prevent cell damage [10–12]. Conversely, when the endothelial monolayer is disrupted or its normal function is perturbed, as for example, by acute or chronic inflammation during atherosclerosis, diabetes, or chronic arterial hypertension, the endothelium becomes proinflammatory [2, 13, 14]. This state is characterized by enhanced expression of growth factors, adhesion and signaling molecules, lipid mediators [15], cytokines, and chemokines controlling recruitment of circulating leukocytes from the blood and lymph to inflammatory sites [16, 17]. Lipid mediators synthesized from essential fatty acids play pivotal roles in distinct phases of the inflammatory response [15]. Thus, prostaglandin (PG)E_2 and cysteinyl leukotrienes (cysLT) promote early vascular permeability and leukotriene (LT)B_4 stimulates leukocyte chemotaxis [18]. PGs play additional roles during the acute inflammatory response, as they regulate local changes in blood flow and pain sensitization [19]. In the case of endothelial disruption, the denuded vessel wall induces thrombin generation leading to a prothrombotic state [13, 14]. Although in each case a distinct set of events is triggered [5, 20, 21], they ultimately culminate in the initiation of coagulation, thrombin formation, and fibrin deposition at the site of injured vascular wall contributing to wound healing and restoration of the hemostatic balance.

Besides being a final protease in the coagulation cascade, thrombin is a very important mitogenic agent. Extensive studies were conducted to elucidate the mechanisms by which thrombin receptors, in particular protease-activated receptor (PAR) 1, couple to the mitogen activated protein kinase (MAPK) signaling cascade. Studies have shown a role of the MAPK-dependent extracellular signal regulated kinase (ERK1/2) pathway in cellular proliferation and migration. Signaling via ERK1/2 pathway depends on activation of epidermal growth factor receptor (EGFR), a well-studied tyrosine kinase receptor [22–24] signaling in a G-protein-coupled receptor independent or dependent fashion [23, 24]. Thrombin is also a potent chemoattractant for monocytes and vascular smooth muscle cells (VSMCs) [25, 26].

Several reports suggested that thrombin predominantly regulates endothelium-dependent vasorelaxation in different species in vitro [27–29]. In addition, Gudmundsdóttir et al. recently showed that thrombin induces PAR1-mediated arterial vasodilatation in humans in vivo [30], effects that were attributed to vasoprotective molecules such as PGE_2 [31, 32], prostacyclin (PGI_2) [31, 33], endothelium-derived hyperpolarizing factor (EDHF) [34], and mainly nitric oxide (NO) [35, 36].

Role of thrombin in vascular physiology

Thrombin was originally identified as a trypsin-like serine protease, that converts soluble fibrinogen into insoluble fibrin [37]. Thrombin is generated through proteolytic cleavage of its inactive precursor, prothrombin, which is synthesized in the liver [38, 39]. In addition to its role in a clot formation, thrombin is also a strong activator of a number of cell types such as endothelial cells (ECs), VSMCs, platelets, and dendritic cells (DCs) [38, 40, 41]. The cellular responses to thrombin are mediated via protease-activated receptors (PAR) 1, 3, and 4 [25], a family of seven transmembrane G-protein-coupled receptors activated by proteolytic cleavage of the amino-terminal extracellular domain [7, 38]. Cleavage of this domain unmasks a new amino terminus that acts as a tethered ligand to autoactivate the receptor [38].

PAR1, the major receptor to which most of the cellular and platelet actions of thrombin are attributed [38, 42, 43], possesses a well-defined role in vascular remodeling and atherosclerosis [30, 44]. However, relatively little is known about the functions of the other thrombin receptors, PAR3 and PAR4, in humans. Vascular PAR3 has been reported to act as a cofactor for PAR1, regulating signaling by receptor dimerization that leads to increased endothelial permeability [45]. Furthermore, in human embryonic kidney cells, PAR3 is able to trigger signals independent from other thrombin receptors [46]. The same authors demonstrated that the thrombin-mediated PAR3 activation results in ERK1/2 phosphorylation and increased production of interleukin (IL)-8 [46]. Vidwan et al. showed that

activation of PAR3 and PAR4 accelerates tissue factor (TF)-induced generation of thrombin on the surface of VSMCs [47]. PAR4 is also reportedly involved in myocardial reperfusion injury [48] and in the endothelial response to inflammatory challenge [49]. Dangwal and colleagues demonstrated that exposure of VSMCs to high glucose enhances thrombin responses via PAR4 inducing tumor necrosis factor (TNF)-α expression and VSMCs migration [50]. Furthermore, PAR1 acts as the major thrombin receptor on human platelets, whereas PAR4 requires higher concentrations of thrombin for activation [24, 51, 52]. It has been suggested that PAR1 accounts for the initial platelet aggregation in response to thrombin, while PAR4 maybe responsible for the stability of platelet aggregation [53]. Indeed, Wu et al. demonstrated that PAR4 is responsible for maintaining the thrombin-induced platelet aggregation [54].

Effects of thrombin on platelets and in wound healing

Platelets are anucleate cells derived from bone marrow megakaryocytes [55], involved in homeostasis, wound healing and inflammation [56, 57]. Under physiological conditions, platelets circulate in quiescent state. Platelets are protected from untimely activation by antithrombotic mediators released from intact ECs, including NO [58, 59] and PGI_2 [60]. However, vascular injury promotes changes in release of antithrombotic mediators that may lead to increased platelet activation followed by their interaction with neutrophils and monocytes [58, 61, 62]. Activation of platelets is associated with changes in cell shape, secretion of granule contents (adenosine diphosphate and serotonin, for example), and engagement of fibrinogen receptor resulting in platelet adhesion and aggregation [61–63]. These events trigger catalytic activity within the vasculature resulting in thrombin generation and formation of a platelet–fibrin clot at the site of injury [24]. Typically, thrombin generation requires series of catalytic reactions regulated by enzymatic complexes assembled on the surface of activated platelets [63]. It is generally accepted that the thrombotic response is initiated during vascular injury (e.g., due to disruption of endothelial cell layer or plaque rupture) [64] when TF expressed either by activated endothelial cells [65], monocytes, VSMCs [66] or subendothelial matrix, but also adventitial fibroblasts [66] interacts with the serine protease factor VIIa (FVIIa) [67–69].

TF is a type-1 integral membrane protein that functions as a cofactor together with FVIIa [70] to activate FX [71]. Activated FXa in concert with cofactor Va converts prothrombin into its active form, thrombin. Thrombin formed on the surface of activated platelets dramatically amplifies the coagulation response via conversion of procofactors V and VIII into active forms Va [72, 73] and VIIIa [74, 75]. Furthermore, an additional coagulation stimulus is provided by additional FIXa generated through the proteolytic activation of FIX by FXIa bound to platelets [76, 77] after the TF–FVIIa reaction has been inhibited by plasma inhibitors [78, 79]. In addition, it has been proposed that vessel wall-derived TF is effectively shielded from contributing to subsequent luminal growth of the thrombus by the diffusion barrier of the thrombus material itself [80, 81]. On the other hand, life-threatening vascular diseases such as acute myocardial infarction and stroke develop due to complete occlusion of blood flow within medium- and large-sized blood vessels [82–84]. Such occlusion is caused by overgrown thrombi despite the fact that classical coagulation pathway maybe inhibited [80, 85]. These observations suggest the existence of an additional mechanism able to propagate thrombus growth. Indeed, several studies have demonstrated that in vivo at injury sites blood-borne TF could be responsible for the thrombus propagation [85–87]. These studies suggested that microparticles might bear TF and P-selectin glycoprotein ligand-1 (PSGL-1, a leukocyte protein). Thus, even when further interactions between vessel wall-derived TF and circulating blood maybe prevented by a mural thrombus itself, a circulating pool of TF could contribute to further thrombus growth [88]. Inflammatory mediators might increase both, the number of microparticles through leukocyte activation and the concentration of TF on the particle surface. As the particles flow over the developing thrombus, they adhere to the thrombus through interaction between the particle membrane surface, rich in TF and PSGL-1-P-selectin [89]. Therefore, the leukocyte adhesion molecule that was originally believed to be mainly involved in leukocyte trafficking appears to play a dominant role also in thrombus development [90].

Once thrombin is generated, it activates platelets to produce a potent lipid mediator, thromboxane A_2 (TXA_2) which recruits even more platelets to the site of injury thereby amplifying thrombus formation [91]. TXA_2 is produced endogenously from phospholipids of the platelet membrane via activated cytosolic phospholipase A_2 ($cPLA_2$) [91]. Aspirin, which prevents generation of TXA_2 thereby impairing platelet activation, has gained widespread recognition as an effective antithrombotic agent [92]. These findings suggest that generated TXA_2 is very important for the maximal platelet activation and maintenance of vascular homeostasis [93]. Thrombin-induced production of TXA_2 is mediated by both thrombin receptors PAR1 and PAR4 [94, 95] and is associated with phosphorylation and activation of $cPLA_2$ [96]. Although, there has been a considerable debate regarding the role of MAPKs, such as ERK1/2 and p38 MAPK in platelet functional responses, several studies reported essential

roles of these kinases in platelet aggregation [97–99], granule secretion [98, 99], thrombus formation [100], and $cPLA_2$ activation [101]. In contrast, other studies seemed to indicate that MAPKs do not contribute to any of these platelet responses [102–104]. However, recent data from the Kunapuli group revealed that ERK1/2 activation is essential for glycoprotein (GP)Ib-mediated TXA_2 generation and $P2Y_{12}$-receptor mediated platelet aggregation [105, 106]. Furthermore, thrombin may also mediate platelet adhesion and activation by binding to GPIbα in addition to PAR1 and PAR4 [107]. GPIbα is a major subunit of the GPIb-IX–V complex, which represents a receptor for von Willebrand factor (vWF) and mediates platelet adhesion and activation [108]. It has been proposed that interaction of thrombin with GPIbα may favor the subsequent proteolytic activation of PAR1 [109], although other studies indicated that stimulation of platelets with thrombin upon desensitization of both PAR-1 and PAR-4 still promotes phosphorylation of MAPKs and activation of the Rho-dependent kinase p160ROCK suggesting an active and direct role of GPIb-IX–V in thrombin-induced transmembrane signaling [110]. Soslau et al. [111], for instance, proposed that binding of thrombin to GPIbα may initiate a new pathway for platelet aggregation that does not involve PARs and is supported by polymerized fibrin. In a line with this observation, Torti's group showed that thrombin induces platelet activation in the absence of functional PAR1 and PAR4 and GPIb-IX–V [112]. These authors demonstrated that thrombin binding to GPIbα induces activation of PLC. The same authors suggested existence of one or more receptors on platelets that transduce signals initiating cell activation followed by induction of tyrosine kinases, cytoskeleton reorganization, integrin αIIbβ3 activation, and aggregation [112].

Wound healing is a biologically complex process comprising three sequential, yet overlapping phases: (i) inflammatory, (ii) proliferative, and (iii) remodeling [113]. The inflammatory phase starts with the coagulation cascade and through series of enzymatic processes leading to thrombin generation and fibrin clot formation [32, 114] aimed to restore the homeostatic balance. Thrombin formed on the surface of activated platelets could amplify the coagulation cascade [72–75] and promote additional recruitment of platelets to the growing thrombus [91]. In turn, activated platelets secrete a wide spectrum of proinflammatory and immune-modulatory molecules, including adhesion molecules (e.g., fibrinogen, vWF, and P-selectin) [115–121], chemokines such are platelet factor 4 (PF4), IL-8, and monocyte chemoattractant protein (MCP)-1 [122–124], coagulation factors such are FV, FXI, and FXIII [63], plasminogen activator inhibitor (PAI)-1, and plasminogen [125–128]. In addition, there are numerous studies demonstrating the role of platelets in proliferative phase of wound healing [129–131]. The spectrum of growth factors secreted by platelets, including VEGF [132, 133], PDGF [134], FGF [135], and TGF-β [136] promotes vessel wall permeability and recruitment, growth, and proliferation of endothelial cells and fibroblasts. Although these growth factors are secreted by a variety of inflammatory cells, the rapidity with which platelets accumulate at sites of vascular injury makes them a relevant source of mitogenic mediators. For example, VEGF concentrations are markedly elevated during the first minutes after plug formation following forearm incision [42]. VEGF also accumulates inside platelet thrombi formed in vivo [137]. Platelet-derived CXCL12 has been reported to induce recruitment of CD34 + progenitor cells to arterial thrombi in vivo and promote differentiation of cultured CD34 + cells to endothelial progenitor cells [138, 139]. Klark et al. demonstrated that preparations that include platelets and platelet supernatant enriched with α granule proteins increase proliferation and migration of osteogenic cells [140]. The same platelet preparation also stimulates proliferation of human tendon cells in culture and promotes significant synthesis of VEGF and HGF [141]. Studies in dogs demonstrated that platelet preparation in a collagen sponge promotes periodontal tissue regeneration [142]. These data indicate that thrombin-mediated platelet accumulation and activation is essential for various vascular processes and maintenance of homeostasis.

Furthermore, thrombin stimulation of VSMC also induces expression of cytokines and cytokine-inducible molecules, including IL-6, IL-8, MCP-1, and IL-1 [143, 144]. Wilcox et al. described increased expression of thrombin receptor mRNA and protein after vascular injury [145]. Besides described proinflammatory effect of thrombin on VSMCs [143, 144], several studies suggested the role of thrombin in inducing VSMCs proliferation, thus linking these cells to complex process of wound healing [121, 146, 147].

Effects of thrombin on dendritic cells and leukocytes

DCs are essential for the induction of the adaptive immune response [148, 149]. On the basis of their phenotype and their ability to prime naive T cells, they are commonly subdivided into immature and mature DCs [150]. Antigens, pathogens, lipopolysaccharide (LPS), and tumor necrosis factor (TNF)-α induce functional changes culminating in the transition from antigen-capturing immature to antigen-presenting mature DCs [149, 150]. Yanagita et al. [151] showed that thrombin stimulation of blood DCs induces cytokine secretion via PAR1. Secreted cytokines in turn could modulate coagulation events or inflammatory responses [152, 153]. Furthermore, thrombin increased the expression of human leukocyte antigen (HLA)-DR and

CD86 on blood DCs and their capacity to stimulate allogeneic T cells to proliferate more efficiently than non-stimulated DCs [151]. Results reported by Yanagita et al. [151] suggest that thrombin plays a very important role in polarizing T cell development. Thus, the interaction between thrombin and PAR-1-expresing blood DCs could play pivotal roles in regulating local inflammatory and immune responses [151]. Furthermore, different maturation stimuli trigger expression of functional thrombin receptors in DCs [41]. It was also shown that in LPS-matured DCs, thrombin induces chemotactic responses and increased release of CCL18 chemokine ligand via PAR1 and PAR3 [41].

Polymorphonuclear neutrophils (PMNs) play an important role in host defense and in the pathogenesis of various diseases [154]. Apart from the classical recruitment of PMNs to inflamed tissues [154], platelets bound to activated endothelium could promote interaction of neutrophils first with platelets, followed by neutrophil-endothelial interaction [155]. PMNs may also modulate activation of blood coagulation through the production and release of reactive oxygen species [156]. Depending on the number of PMN and the amount of reactive oxygen species produced by them, the expression of TF by coincubated mononuclear cells was either positively or negatively regulated. Moreover, it has been suggested that PMNs themselves can be induced to express TF [157, 158]. In contrast to PMNs that do not support the assembly and function of intrinsic tenase [159], mononuclear cells can recruit FVa and FXa assembling a functional prothrombinase complex that is analogous to that expressed by activated platelets and monocytes [160, 161], and that displays a catalytic efficiency identical to that expressed by activated platelets and monocytes [161].

Prolonged stimulation of monocytes with cytokines released at the site of injury [162] combined with P-selectin expression by activated platelets [163] will induce synthesis and expression of functional prothrombinase and TF [160] on the monocyte surface. These two important factors of the coagulation cascade were shown to possess equally potent catalytic efficacy than that expressed on activated platelets. Because the TF activity can be inhibited by coexpression of TF pathway inhibitor, the ability of monocytes to generate factor Xa via intrinsic tenase could be critical for sustained thrombin generation at the monocyte surface [164].

Thrombin and vascular inflammation

There is an extensive cross-talk between inflammation and coagulation, whereby inflammation leads to activation of coagulation, which, in turn, considerably affects the inflammatory process [90, 165]. Indeed, activation of the coagulation cascade with the formation of thrombin as a key effector protease, creates a proinflammatory environment affecting the endothelium and the innate immune cells in particular [165]. Thrombin activates platelet aggregation and has direct effects on monocytes [166–168], VSMCs [143, 169–171], ECs [172–178], lymphocytes [38, 179, 180], and DCs [41, 181]. In addition, thrombin is mitogenic for VSMCs [146, 182] and fibroblasts [183, 184], and chemotactic for monocytic cells [185]. Furthermore, thrombin triggers a wide spectrum of endothelial responses, such as the production of prostacyclin [186], platelet-activating factor [187, 188], endothelin [189, 190], von Willebrand factor [191–193], and plasminogen activator [20, 194, 195] and its inhibitor [196]. In response to thrombin, cultured endothelial cells also secrete enhanced levels of PDGF [197, 198], which is a potent mitogen and chemoattractant for VSMCs [199]. Expression of very important proatherogenic adhesion molecules, which facilitate emigration of leukocytes from the vessels, such as vascular cell adhesion molecule-1 (VCAM-1), intracellular cell adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin are also increased by thrombin [173, 175, 186, 200–203]. In addition, thrombin-stimulated ECs show increased permeability [200] as well as recruitment and migration of leukocytes across the endothelium [175] in response to diverse chemoattractants, which requires various integrins and cell adhesion glycoproteins (Fig. 1).

Impaired homeostasis and increased cellular adhesion lead to endothelial dysfunction that is thought to be a prerequisite for the initiation of an atherosclerotic plaque. Indeed, increased thrombin generation was observed in patients with advanced cardiovascular disease and acute coronary syndrome [204]. Furthermore, we have previously demonstrated the possible interaction between thrombin-stimulated ECs and monocytes [175], by showing that MCP-1 synthesis in monocytes co-cultured with ECs is mediated by thrombin-induced expression of fractalkine, a chemokine that potently attracts T cells and monocytes and has a definite role in the progression of cardiovascular disease [175].

Some of the pro-inflammatory features of thrombin have been inferred from models of inflammation such as a murine model of peritonitis. In this model, administration of the potent inhibitor of thrombin, hirudin, inhibited antigen- or lipopolysaccharide (LPS)-stimulated activation of macrophage adhesion [188]. Further in the same model, administration of purified thrombin- stimulated adhesion of macrophages and overexpression of IL-6 and MCP-1 in a fibrinogen-dependent and PAR1-independent fashion [205]. Yet another important role of thrombin has been demonstrated in murine heart-to-rat xenotransplantation model. In this model, the recruitment of monocytes and

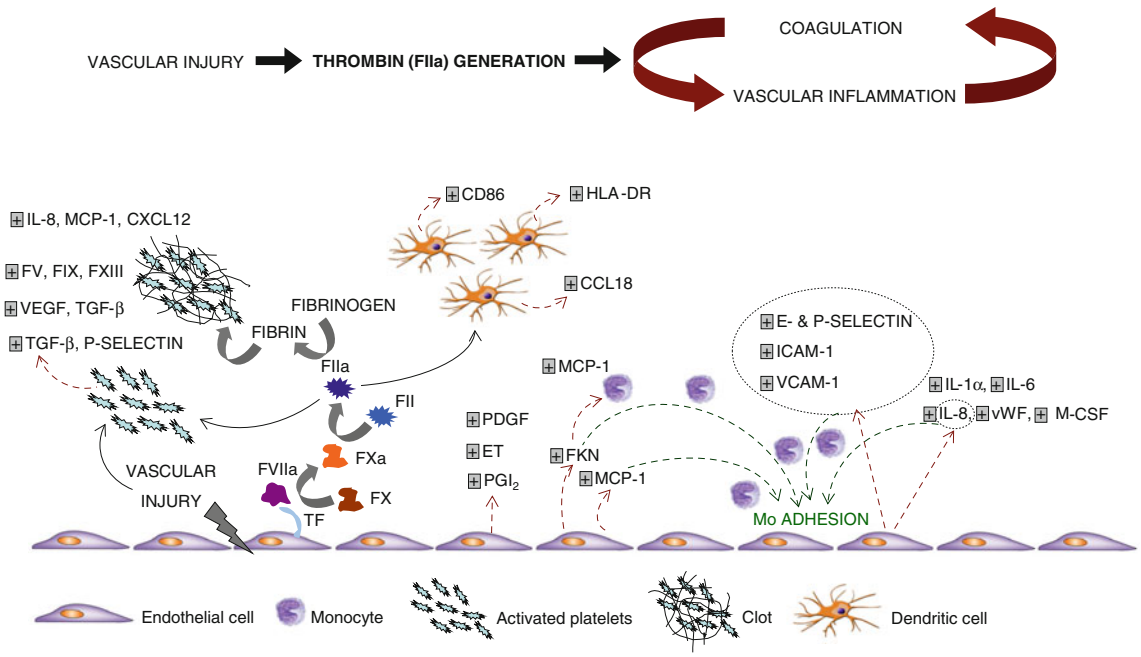

Fig. 1 Proposed mechanisms of the contribution of thrombin to vascular inflammation. *Square with plus* symbol indicates induction; *upward arrows* indicate elevated levels; *downward arrows* indicate adhesion initiation; *HLA-DR* human leukocyte antigen-DR; *VEGF* vascular endothelial growth factor; *TGF-β* transforming growth factor-*β*; *PAI-1* plasminogen activator inhibitor-1; *CCL18* chemokine (C–C motif) ligand 18; *CXCL12* chemokine (C-X-C motif) ligand 12; *PDGF* platelet-derived growth factor; *ET* endothelin; PGI_2 prostacyclin; *FKN* fractalkine; *MCP-1* monocyte chemoattracant protein-1; *Mo* monocyte, *VCAM-1* vascular cell adhesion molecule-1; *ICAM-1* intercelullar adhesion molecule-1; *IL* interleukin; *M-CSF* macrophage-colony-stimulating factor; *vWF* von Willebrand factor; *TF* tissue factor; *FXIII, XI, VII, II* factor X, VII, II; *Xa*, *FXa, VIIa, IIa* activated factor X, VII, II

natural killer cells to the graft in vivo has been attributed to a thrombin-mediated activation of PAR1 leading to local generation of MCP-1 [206].

As mentioned earlier, thrombin is known to potentiate the production of IL-6 in both, ECs [207] and VMSCs in vitro [208]. IL-6 is an important molecule with a well-established role in inflammation and is reported to exacerbate atherosclerosis [209]. Expression of IL-8 in endothelium is also induced by thrombin via the p38 MAPK signaling pathway in vitro [185], and IL-8 may trigger monocyte adhesion to endothelium under flow conditions in vitro [210]. In addition, thrombin induces secretion of macrophage migration inhibiting factor in ECs and VSMCs [211].

Despite the abundance of available data on thrombin's proatherogenic actions in vivo, many of these results, however, have been inferred from cell cultures using purified thrombin, in the absence of natural inhibitors. Hence, the relevance of those studies with respect to systems biology is questionable. However, in vivo studies clearly supported the critical role of thrombin in atherogenesis [212–214]. Studies employing transgenic double knock-out mice deficient for the natural inhibitor of thrombin, heparin-cofactor II, a on a $ApoE^{-/-}$ background showed significantly increased plaque areas and increased neointimal formation when compared with wild-type mice [215]. Furthermore, a recent murine study with CX3CL1/CCR2/apoE triple-knockout mice provided evidence for independent roles of CCL2 and CX3CL1 in terms of macrophage accumulation and atherosclerotic lesion formation [214].

Thus, the diverse cellular responses triggered by thrombin may contribute to the pathology of atherosclerosis, thrombosis, and vasculitis through inflammatory and proliferative responses at sites of vascular injury [25, 35, 216].

Conclusion

The concept of an extensive cross-talk between inflammation and coagulation has been established in the past several years [90, 165]. Vascular inflammation leads to activation of coagulation and, in turn, coagulation considerably affects the inflammatory process [56, 217]. Indeed, activation of the coagulation cascade with the formation of thrombin as a key protease, creates a proinflammatory environment affecting the endothelium and innate immune cells in particular [165]. Thrombin accomplishes the majority of its actions including multiple vascular proinflammatory responses, via PARs [25, 31, 33]. When injury of the blood vessel wall causes disruption of its endothelial layer, activation of the coagulation cascade is required as a

part of natural healing process [90, 218]. Thrombin formed on the surface of activated platelets would amplify the coagulation cascade [72–75] and promotes additional recruitment of platelets to the growing thrombus [91]. In turn, activated platelets produce and secrete a wide spectrum of proinflammatory and immunomodulatory molecules contributing to inflammatory as well as healing processes [63, 115–131].

Teleologically, the coagulation process is intended to prevent blood loss and to initiate wound healing. However, platelets activated during this process may well contribute to the inflammatory response [56, 217]. Thrombin, generated on the surface of activated platelets, obviously amplifies the process of coagulation, but also stimulates platelets as well as other cell types such as ECs, VSMCs or leukocytes to secrete a broad spectrum of bioactive molecules with distinct roles in coagulation and inflammation. Thus, thrombin and its subsequent signaling takes center stage as an important pharmacotherapeutic target in vascular homeostasis. More recent approaches encompass antiplatelet therapies involving antibodies directed against thrombin receptors [219–222]. Further studies on potential novel therapies might focus on the regulation of thrombin actions through mechanisms different from PARs. Better knowledge of thrombin-induced signaling still holds great promise for improved and novel therapeutic applications.

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