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The many faces of tissue factor

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Summary

Tissue factor (TF) is a member of the cytokine receptor superfamily and binds FVII/VIIa. The TF:FVIIa complex has both procoagulant and signaling activities. It functions in many biological processes, including hemostasis, thrombosis, inflammation, angiogenesis and tumor growth. Importantly, TF is essential for hemostasis. However, increased TF expression within atherosclerotic plaques and elevated levels of circulating TF-positive micro particles promote thrombosis. TF increases inflammation by enhancing intravascular fibrin deposition, by increasing the formation of pro-inflammatory fragments of fibrin and by generating coagulation proteases, including FVIIa, FXa and thrombin, that activate protease-activated receptors (PARs). In endotoxemia and sepsis, TF-dependent thrombin generation and activation of PAR1 on dendritic cells enhance inflammation. Finally, the TF:FVIIa complex contributes to tumor growth by activating PAR2.

Keywords

coagulation; hemostasis; inflammation; review; thrombosis

Introduction

TF plays a role in many biological processes. However, a major challenge to studying TF in these processes is that knock-out of the TF gene in mice is lethal and inhibition of TF can induce hemorrhage. However, these problems have been overcome with the development of pharmacologic tools and genetic approaches. For instance, a monoclonal antibody called 10H10 was identified that inhibits TF-dependent signaling without affecting TF procoagulant activity. This antibody can be used to study TF signaling without affecting hemostasis. To circumvent the problem of embryonic lethality, we made mice ('low-TF' mice) that express low levels of TF in all tissues (1% of wild-type levels). In addition, we made mice that have the TF gene deleted in a cell type-specific manner, including in myeloid cells ($TF^{flox/flox}$, LysMCre) and in vascular smooth muscle cells ($TF^{flox/flox}$, SM22Cre). Because of space limitations, some primary references could not be included in the review.

Hemostasis

TF is expressed by cells in the blood vessel wall and activates the clotting cascade after vascular injury (Fig. 1). A recent study showed that vessel wall TF is pre-bound with FVII [1]. TF is also expressed in a tissue-specific manner [2]. Interestingly, either a genetic deficiency of TF

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Disclosure of Conflict of Interests

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expression or inhibition of TF in wild-type mice leads to tissue-specific hemorrhage in tissues with high levels of expression, such as the brain and heart. This suggests that TF-dependent thrombin generation is required for hemostasis in vital organs.

The TF:FVIIa complex also participates in 'idling' of the clotting cascade. This is defined as a low basal activation of clotting that occurs in healthy individuals [3]. Hematopoietic cell-derived TF appears to mediate idling because the basal level of thrombin–antithrombin (TAT) complex in plasma is decreased in wild-type mice containing bone marrow from low TF mice [4].

Thrombosis

Pathologic TF expression can trigger arterial thrombosis, venous thrombosis and disseminated intravascular coagulation (DIC) (Fig. 1). In contrast to the high levels of TF in the vessel wall, only very low levels of TF are present in blood of healthy individuals. This so-called 'circulating TF' consists mainly of TF-positive micro particles (MP), which are submicron membrane fragments derived from activated and/or apoptotic cells. Unstimulated monocytes express low levels of TF and may be the primary source of these MPs. Importantly, levels of TF-positive MP are increased in a variety of diseases, including cardiovascular disease, sepsis and cancer [5]. These TF-positive MP most likely contribute to thrombosis.

In humans, atherosclerotic plaques contain large amounts of TF and this triggers thrombosis after plaque rupture. In healthy mice, vessel wall TF mediates the formation of an occlusive thrombus in a carotid artery injury model [6]. In contrast, both vessel wall-and hematopoietic cell-derived TF contribute to thrombosis in a laser-induced injury model of mouse cremaster arterioles [7]. Recently, we used $TF^{flox/flox}$, SM22Cre mice to show that deletion of the TF gene in vascular smooth muscle cells in mice reduced carotid arterial thrombosis [8].

Venous thrombosis is triggered by a combination of factors that include increased thrombogenicity of blood. As venous thrombosis is generally not associated with vessel-wall damage, it is likely that circulating TF rather than vessel-wall TF triggers venous thrombosis. One study showed that MPs isolated from pericardial blood of cardiac surgery patients promoted TF-dependent venous thrombosis in a rat model [9]. However, we found that in healthy mice hematopoietic cell-derived TF did not contribute to venous thrombosis in an inferior vena cava ligation model [6].

Cancer patients exhibit a high rate of venous thrombosis. A recent retrospective study found elevated levels of TF-positive micro-particle activity in plasma from patients with breast and pancreatic cancer [10]. In a prospective study, we found that pancreatic cancer patients with elevated levels of TF-positive micro-particle activity developed venous thromboembolism [11]. These studies suggest that circulating TF may contribute to venous thrombosis in cancer patients. Moreover, levels of TF-positive micro-particle activity may be a useful biomarker to identify cancer patients with an increased risk of thrombosis. Other studies have analyzed levels of circulating TF in mouse tumor models. We found that human colorectal tumors grown in mice release human TF antigen into the circulation [12]. Another study found a positive correlation between levels of plasma TAT complex and tumor-derived human TF activity from mice containing human pancreatic tumors [13]. These results suggest that circulating tumor-derived TF activates the clotting cascade in mice and may promote venous thrombosis.

Inhibition of the TF:FVIIa complex with a variety of inhibitors reduces DIC in animal models of endotoxemia and sepsis. *In vitro* and *in vivo* studies have shown that lipopolysaccharide (LPS) released from Gram-negative bacteria is a potent inducer of TF expression in monocytes [5]. In a human endotoxemia model, monocytes express TF and release TF-positive micro-particles into the circulation [5]. We found that reducing TF expression in hematopoietic cells

decreased levels of TAT complex in a murine endotoxemia model [4]. Similarly, we used $TF^{flox/flox}$, LysMCre mice to show that deletion of the TF gene in myeloid cells decreases levels of TAT complex after administration of LPS (Pawlinski and Mackman, unpublished data). In septic baboons, TF antigen is also observed on the surface of endothelial cells at branch points of the aorta [14]. However, this TF antigen may be attributable to the deposition of leukocyte-derived, TF-positive micro-particles on the activated endothelium. In support of this notion, a recent study showed that TF staining of neutrophils isolated from LPS-stimulated human whole blood was caused by binding of monocyte-derived, TF-positive micro-particles [15]. These results indicate that monocytes are the major source of inducible intravascular TF that leads to DIC in endotoxemia and sepsis.

Inflammation

Many studies have shown that TF contributes to inflammation in a variety of disease models, including endotoxemia, sepsis and ischemia-reperfusion (I/R) (Fig. 1) [16]. For instance, inhibition of TF in a baboon model of sepsis reduced circulating levels of the pro-inflammatory cytokines IL-6 and IL-8, and mortality. In contrast, an active site inhibited FXa molecule reduced DIC without reducing mortality. We found that low TF mice had reduced levels of TAT and IL-6 compared with control mice in an endotoxemia model [4]. These studies suggested that TF-dependent signaling enhances inflammation during endotoxemia. Recently, it was reported that endotoxemic $PAR1^{-/-}$ mice expressed lower levels of IL-6 and exhibited reduced mortality compared with wild-type littermate controls [17]. Inhibition of thrombin in wild-type mice was also protective. Further studies indicated that TF-dependent thrombin generation and PAR-1 signaling in dendritic cells amplifies both inflammation and coagulation [16].

TF also enhances inflammation associated with I/R injury. Neutrophils contribute to reperfusion injury by releasing oxygen radicals and inflammatory mediators. We showed that inhibition of TF in a rabbit model of cardiac I/R injury reduced the expression of inflammatory mediators, reduced recruitment of neutrophils and also reduced infarct size [18]. Inhibition of thrombin provided a similar protection. Thrombin may increase infarct size either by activating PAR1 and/or by enhancing fibrin deposition. Surprisingly, we found that PAR1 deficiency did not affect infarct size [19]. In contrast, a deficiency of fibrinogen was associated with a decrease in infarct size [20]. Further studies demonstrated that the E1 fragment, which is derived from degradation of fibrin, facilitates the recruitment of neutrophils into the myocardium by binding to VE-cadherin at endothelial cell junctions and the integrin CD11c/CD18 on neutrophils [16].

TF may also increase infarct size by activating PAR2. A recent study showed that active site-inhibited FVIIa reduced inflammation and infarct size in a mouse model of cardiac I/R injury [21]. We found that $PAR2^{-/-}$ mice had smaller infarcts than wild-type littermates (Pawlinski and Mackman, unpublished data). However, further studies are required to determine the role of the TF:FVIIa-PAR2 signaling pathway in cardiac I/R injury.

Importantly, different TF-dependent pathways appear to enhance inflammation in different models (Fig. 1) [16]. For instance, we found that $PAR1^{-/-}$ mice, but not $PAR2^{-/-}$ mice, have reduced inflammation in a kidney I/R model, suggesting that a TF-thrombin-PAR1 pathway drives inflammation in this model [22]. In another study, inhibition of TF reduced inflammation in a model of colitis [23]. In antiphospholipid syndrome (APS), complement C5a induces TF expression on neutrophils [24]. In collaboration with Dr Girardi, we found that injection of an antiphospholipid antibody into pregnant mice induced TF expression on neutrophils and PAR2-dependent generation of reactive oxygen species and fetal loss [25]. Further studies are

needed to identify the cell types that express TF in different pathologic settings and how TF-dependent pathways enhance inflammation.

Tumor growth and metastasis

TF is expressed by tumor cells and plays a role in metastasis by activating the clotting cascade. Early studies indicated that it also enhanced tumor growth and angiogenesis. However, these studies were largely descriptive and it was not until recently that a direct role for TF in tumor growth was shown. In collaboration with Dr Rak, we found that knock-down of TF expression with short interfering RNA (siRNA) in colorectal cancer cells dramatically reduced tumor growth in mice but did not affect tumor growth *in vitro* [12]. Other studies have shown that pharmacologic inhibition of the TF:FVIIa complex reduces tumor growth in mice [26–28]. The most likely explanation for these results is that TF on the surface of tumor cells binds plasma FVIIa and that this cleaves PAR2. In support of this notion, selective inhibition of TF:FVIIa signaling using 10H10 reduced breast tumor growth in mice [29]. Finally, in a mouse model of spontaneous mammary tumors a deficiency of PAR2, but not PAR1, reduced tumor growth [30]. The precise mechanism by which the TF:FVIIa-PAR2 pathway increases tumor growth is not known but possibilities include enhancing tumor survival and/or increasing angiogenesis. TF-dependent thrombin generation and activation of PAR1 on tumor cells may also increase the growth of some tumors.

Conclusion

This review shows that TF plays a critical role in a diverse set of biological processes. However, further studies are required to address several unresolved issues: (i) Does circulating TF contribute to the propagation of a hemostatic clot in healthy individuals? (ii) Do TF-positive micro-particles induce venous thrombosis and can it be used as a biomarker of thrombotic risk? (iii) What is the role of platelet TF? and (iv) What is the relative importance of phosphatidlyserine exposure vs. oxidation of the Cys186/Cys208 pair in the regulation of TF activity in different cell types?

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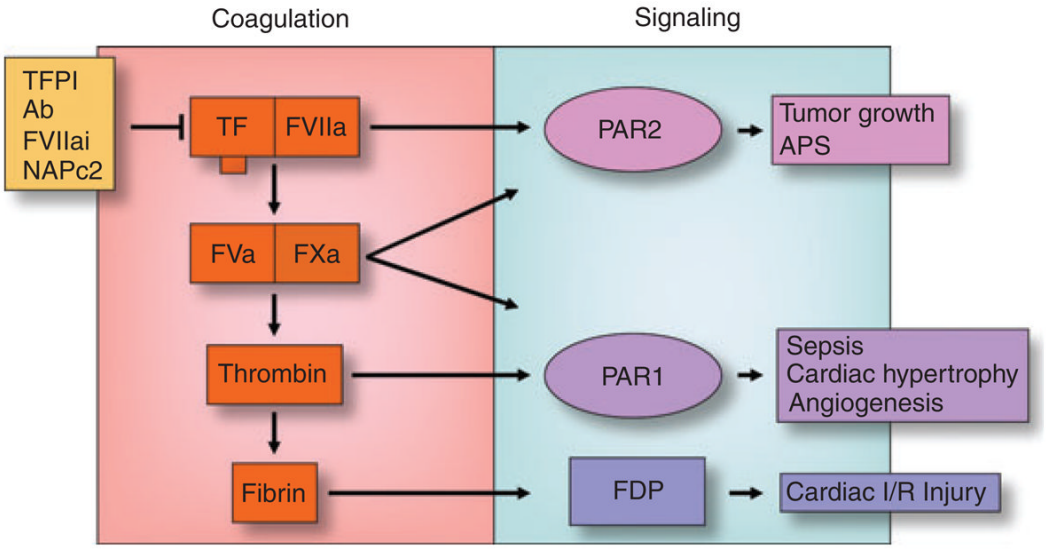

Fig. 1.

TF-dependent procoagulant and signaling pathways. TF plays an essential role in coagulation by generating coagulation proteases and inducing fibrin deposition. Non-coagulant roles of TF in other biological processes include the formation of fibrin degradation products (FDP) and the generating coagulation proteases that activate PAR1 and PAR2. Thrombin also activates PAR3 and PAR4 (not shown).