

Role of the Extrinsic Pathway of Blood Coagulation in Hemostasis and Thrombosis

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Abstract—Hemostasis requires both platelets and the coagulation system. At sites of vessel injury, bleeding is minimized by the formation of a hemostatic plug consisting of platelets and fibrin. The traditional view of the regulation of blood coagulation is that the initiation phase is triggered by the extrinsic pathway, whereas amplification requires the intrinsic pathway. The extrinsic pathway consists of the transmembrane receptor tissue factor (TF) and plasma factor VII/VIIa (FVII/FVIIa), and the intrinsic pathway consists of plasma FXI, FIX, and FVIII. Under physiological conditions, TF is constitutively expressed by adventitial cells surrounding blood vessels and initiates clotting. In addition so-called blood-borne TF in the form of cell-derived microparticles (MPs) and TF expression within platelets suggests that TF may play a role in the amplification phase of the coagulation cascade. Under pathologic conditions, TF is expressed by monocytes, neutrophils, endothelial cells, and platelets, which results in an elevation of the levels of circulating TF-positive MPs. TF expression within the vasculature likely contributes to thrombosis in a variety of diseases. Understanding how the extrinsic pathway of blood coagulation contributes to hemostasis and thrombosis may lead to the development of safe and effective hemostatic agents and antithrombotic drugs. (*Arterioscler Thromb Vasc Biol.* 2007;27:1687-1693.)

Key Words: coagulation ■ arterial thrombosis ■ deep vein thrombosis

The hemostatic system maintains blood in a fluid state under normal conditions and responds to vessel injury by the rapid formation of a clot. Disruption of the endothelium exposes platelets to collagen in the vessel wall and plasma factor VII/VIIa (FVII/FVIIa) to extravascular tissue factor (TF; Figure 1). Other proteins, such as von Willebrand factor (vWF), facilitate the binding of platelets to the injured vessel wall. The TF:FVIIa complex is traditionally referred to as the extrinsic pathway and is proposed to be the primary activator of the coagulation protease cascade in vivo. Subsequently, propagation of the thrombus involves recruitment of additional platelets and amplification of the coagulation cascade by the intrinsic pathway of blood coagulation, which includes the hemophilia factors FVIII and FIX (Figure 1). Importantly, platelets play a critical role in the amplification of the coagulation cascade by providing a thrombogenic surface. Finally, fibrin stabilizes the platelet-rich thrombus (Figure 1). This review focuses on the role of the extrinsic pathway (TF and FVIIa) in hemostasis and thrombosis.

the subendothelial layers of the vessel wall. TF is constitutively expressed by certain cells within the vessel wall and cells surrounding blood vessels, such as vascular smooth muscle cells, pericytes, and adventitial fibroblasts.¹⁻⁵ TF is also expressed in a tissue-specific pattern with high levels in the brain, lung, kidney, heart, testis, and placenta.⁶⁻⁸ In particular, TF is expressed by astrocytes in the brain, epithelial cells in the lung, cardiomyocytes in the heart, and trophoblasts in the placenta.^{1-3,6,7,9} This distribution of TF is consistent with its essential role in hemostasis.

Humans with severe FVII deficiency (less than 1% of normal plasma levels) are found at a low frequency (1 in 500 000).¹⁰ These patients experience abnormal soft tissue, intraarticular, and mucocutaneous bleeding analogous to patients with hemophilia A or B, who are deficient in FVIII and FIX, respectively. In contrast, humans with a deficiency in TF have not been identified. Consistent with this observation, mice lacking either TF or FVII die during embryonic development or during the perinatal period because of both vascular and hemostatic defects.¹¹⁻¹⁴ To analyze the role of TF in hemostasis, we generated so-called “low TF” mice that express very low levels of human TF from a minigene (about 1% of normal levels) in the absence of murine TF.¹⁵ In addition, Rosen and colleagues made mice that expressed low levels of murine FVII.¹⁶ In support of the notion that the

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TF and FVII in Hemostasis

Hemostasis is the protective physiological response to vascular injury that results in exposure of blood components to

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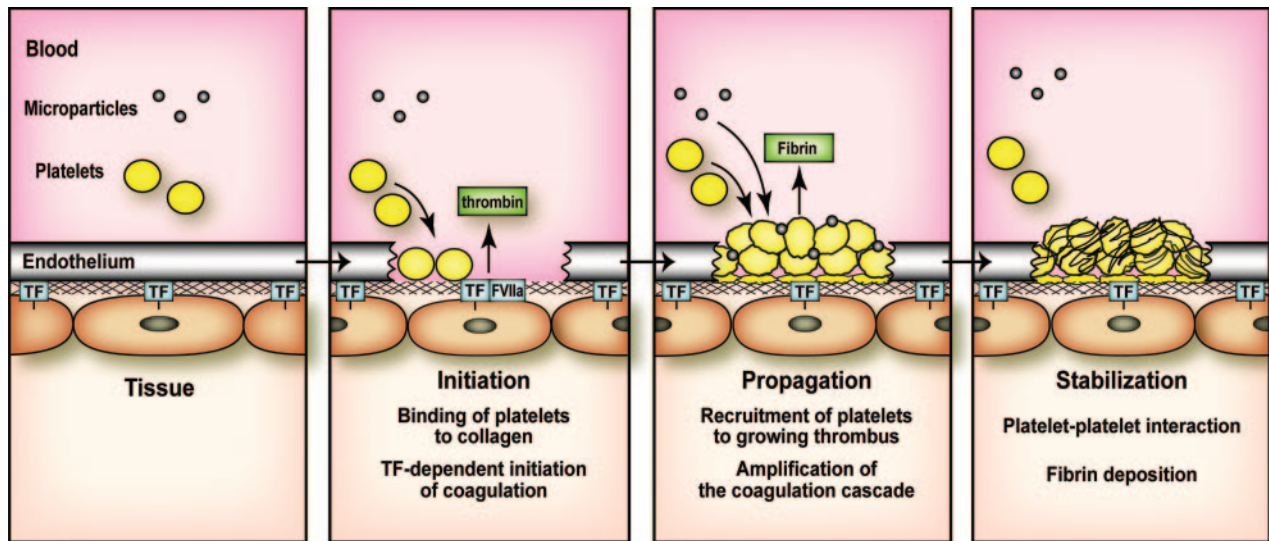


Figure 1. Formation of a clot at the site of blood vessel injury. In a healthy individual, TF expressed by vascular smooth muscle cells, pericytes, and adventitial fibroblasts in the vessel wall is physically separated from its ligand FVII/FVIIa by the endothelium. Vessel injury leads to the rapid binding of platelets to the subendothelium and activation of the coagulation cascade by TF. Propagation of the thrombus involves recruitment of additional platelets and amplification of the coagulation cascade by the intrinsic pathway, and possibly by TF-positive MPs and TF stored in platelets. Finally, fibrin deposition stabilizes the clot. De novo synthesis of TF by platelets may also play a role in stabilization of the clot.

TF:FVIIa complex plays an essential role in hemostasis, low TF and low FVII mice are prone to spontaneous hemorrhages in the lung, heart, and placenta.^{7,15–19} More recently, we demonstrated that cardiac myocyte-specific overexpression of TF restored hemostasis in the hearts of low TF mice (Pawlinski et al, in press), indicating that cardiac myocytes participate in hemostasis. These results from both humans and mice demonstrate that the extrinsic pathway of coagulation is essential for hemostasis.

Recombinant FVIIa and Hemostasis

Clinical studies demonstrate that high levels of recombinant human FVIIa, known commercially as NovoSeven, restore hemostasis in hemophilia A and B patients.^{20,21} Similarly, expression of a mutant version of murine FVII, which is secreted as FVIIa, restores hemostasis in hemophilia B mice.²² NovoSeven has also been used to treat hemorrhages in patients with congenital platelet dysfunction disorders, such as Glanzmann thrombasthemia.^{20,23} Interestingly, administration of NovoSeven is infrequently associated with thrombosis,²⁴ which led to the proposal that NovoSeven can be used as a “universal hemostatic agent” to treat a variety of hemorrhagic disorders.²⁵ However, recent literature analyses have concluded that this concept may be premature.²⁶

The mechanism by which NovoSeven restores hemostasis is not entirely clear.²⁷ Hoffman and colleagues²⁸ proposed a TF-independent mechanism in which NovoSeven binds to the surface of activated platelets thereby localizing the activation of coagulation to sites of vessel injury. In contrast, Mann and colleagues²⁹ concluded that the efficacy of NovoSeven in hemophilia blood is dependent on TF. In vitro studies have shown that FVIIa binding to activated platelets is TF-independent and that FVIIa activates FIX, FX, and FXI in the presence of platelets.^{30,31} These results suggest a model in which FVIIa amplifies the coagulation cascade by activation

of the intrinsic pathway or by direct activation of FX. However, recent studies have shown that TF-positive microparticles (MPs) are incorporated into a growing thrombus and that activated platelets express TF (see below). These results suggest that TF is present in a growing thrombus and may contribute to the hemostatic effects of NovoSeven in vivo.

TF and FVIIa in Thrombosis

Thrombosis may result from a pathologic response to vessel wall injury. This injury, which is usually nontraumatic in nature, may or may not result in exposure of blood to the subendothelium. Under physiological conditions, vascular cells that are in contact with blood do not express TF. In contrast, pathologic conditions lead to induced TF expression by a variety of vascular cells, and this expression plays an important role in thrombosis.³²

TF Expression by Leukocytes

TF expression has been shown to contribute to disseminated intravascular coagulation in a baboon model of sepsis.³³ A key cell type that can be induced to express TF is the monocyte. Indeed, many studies have shown that exposure of monocytes to bacterial lipopolysaccharide (LPS) induces TF expression both in vitro and in vivo.^{1,34,35} One study showed that monocytes expressed TF mRNA in a human model of endotoxemia.³⁶ In contrast, LPS does not induce TF expression in neutrophils and lymphocytes.¹ TF-positive neutrophils were observed in a murine endotoxemia model but no TF mRNA was detected, suggesting that the cells may take-up TF-positive MPs.³⁷ We showed TF expression by hematopoietic cells plays a key role in intravascular coagulation in a murine endotoxemia model.³⁸ More recently, we found that deletion of the TF gene in myeloid cells also reduces LPS-induced coagulation in mice (Tilley et al, unpublished data 2007).

Although there has been some debate about whether or not neutrophils express TF,^{39,40} several recent studies demonstrated robust TF expression by neutrophils in different disease states.^{41–44} For instance, it was shown that antiphospholipid antibody-induced complement activation and generation of C5a-induced TF expression on neutrophils.⁴⁴ This suggested that neutrophils may initiate thrombosis in patients with antiphospholipid syndrome. We found that administration of an antiphospholipid antibody to pregnant mice induced TF expression on neutrophils in a C5a and C5a receptor-dependent manner (Redecha et al, in press). TF expression has also been observed in human eosinophils.³² These studies in both humans and animal models indicate that TF expression by leukocytes plays an important role in thrombosis associated with a variety of diseases.

TF Expression by Endothelial Cells

TF expression is induced in cultured endothelial cells (ECs) exposed to a variety of agents.⁴⁵ However, whether TF is expressed by the endothelium *in vivo* is more controversial. Drake and colleagues⁴⁶ used a highly sensitive immunohistochemical procedure to show that TF was present on ECs of the microvasculature of the spleen but not ECs in other tissues. More recently, TF protein was observed on ECs at branch points in the aorta of septic baboons.⁴⁷ In a rat model of LPS-induced disseminated intravascular coagulation, TF antigen was detected on monocytes but not ECs of the microvasculature of the lung.⁴⁸ TF antigen has also been observed on circulating ECs in patients with sickle cell disease and on ECs of the pulmonary vein in a mouse model of sickle cell disease.^{49,50} It has been reported that tumor endothelium expresses TF, although this has not been observed by all investigators.^{51–53} Other studies have found TF antigen on ECs in patients with atherosclerosis, tuberculosis, and idiopathic inflammatory bowel disease.^{54–56} Finally, TF was detected on ECs of cardiac vessels in rat models of angiotensin II-induced cardiac vasculopathy and cardiac allograft vasculopathy.^{57,58}

Most of these studies, however, cannot distinguish between TF expression by the ECs themselves versus the binding of TF-positive MPs derived from other cell types. We analyzed the functional role of EC TF in LPS-induced coagulation in a murine model by selectively inhibiting nonhematopoietic cell TF in mice expressing human TF on nonhematopoietic cells and murine TF on hematopoietic cells. Inhibition of TF expression by nonhematopoietic cells, which is likely to be primarily attributable to expression on ECs, significantly reduced coagulation, indicating that these cells express functional TF (Tilley et al, unpublished data 2007). These results suggest that the endothelium can express TF *in vivo* and may be an important source of TF that contributes to thrombosis in various diseases.

TF Expression by Platelets

TF expression by platelets has been somewhat controversial. An early study by Engelmann and colleagues⁵⁹ showed that platelets isolated from collagen-stimulated whole blood contained functional TF. This group also detected TF in α -granules of resting platelets that was exposed on the cell

surface after platelet activation.⁶⁰ Another study found that TF associated with the platelet surface was inactive but released TF was functionally active.⁶¹ In contrast, Mann and colleagues⁶² found no detectable TF antigen or activity on quiescent or ionophore-stimulated platelets. Similarly, Osterud and colleagues⁶³ failed to detect TF activity in collagen-activated platelets.

Recent studies have helped to resolve the controversy of whether or not platelets express TF. We analyzed TF mRNA, antigen, and activity in leukocyte-depleted human platelets from normal donors. We did not observe TF mRNA in quiescent platelets but found significant levels of TF mRNA, antigen, and activity in platelets activated with various agonists.⁶⁴ Surprisingly, we demonstrated that platelets contain a stored TF pre-mRNA that is spliced into TF mRNA after platelet activation. It is notable that the splicing of the TF pre-mRNA, at least *in vitro*, is relatively slow compared with the rapid formation of a thrombus. Other studies found that quiescent platelets express variable levels of TF mRNA.^{65,66} Furthermore, TF mRNA levels and TF activity were increased after platelet activation and platelets were shown to synthesize TF protein.⁶⁶ However, the early increase in TF activity after platelet activation was insensitive to protein synthesis inhibitors, suggesting that platelets contain stored TF that is rapidly translocated to the cell surface. The basal levels of TF mRNA observed in quiescent platelets may be attributable to some degree of activation of the platelets *in vivo* or during isolation.

Recently, we showed that platelets from septic patients contain TF mRNA, which indicates that splicing of TF pre-mRNA can occur *in vivo* (Schwertz et al, unpublished data, 2007). These results indicate that platelets have the capacity to bind TF-positive MPs, store TF in α -granules, and to synthesize TF *de novo* (Figure 2). The fact that platelets can express TF dramatically changes our view of the regulation of blood coagulation because it suggests that TF may contribute to both initiation and amplification of the clotting cascade. However, further studies are needed to determine the physiological role of platelet TF in hemostasis and thrombosis.

Circulating TF-Positive Microparticles

TF antigen has been detected in human platelet-free plasma.⁶⁷ The majority of this TF is present in the form of MPs, which are small membrane fragments released from activated or apoptotic vascular cells.⁶⁸ Importantly, levels of TF-positive MPs are elevated in patients with a variety of diseases, including cardiovascular disease, diabetes, cancer, sickle cell disease, and endotoxemia.^{67,69–73} A recent study showed that patients with disseminated breast and pancreatic cancer had significantly increased levels of MP-associated TF activity compared with nonmetastatic cancer patients.⁷¹ This has led to the suggestion that these TF-positive MPs contribute to thrombosis in these patients. Therefore, pharmacological inhibition of the release of these TF-positive MPs may represent a novel strategy to reduce the risk of thrombosis.

Many cell types can generate circulating TF-positive MPs. For instance, leukocytes, ECs, platelets, and vascular smooth muscle cells have all been shown to produce TF-positive

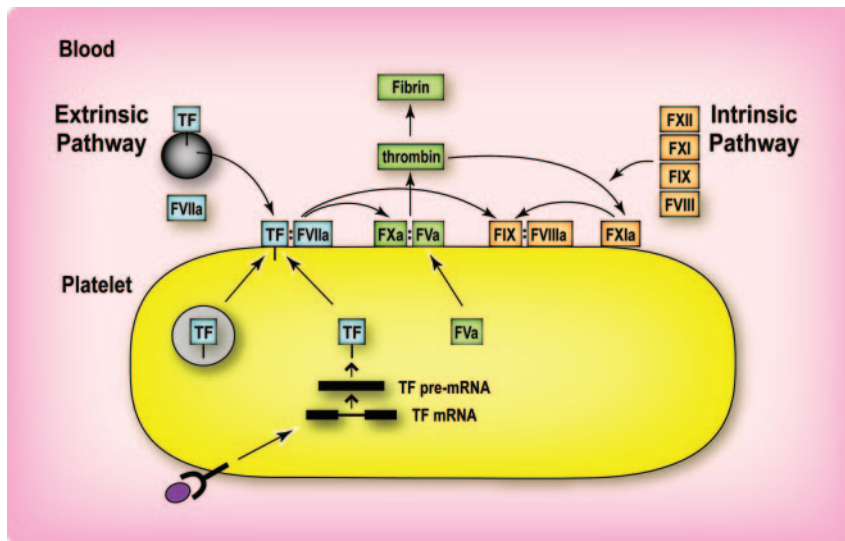


Figure 2. Potential role of platelets and the different coagulation pathways in the generation of thrombin and fibrin. Platelets have 3 sources of TF: MP TF, TF stored in α-granules, and de novo synthesized TF. These different sources participate in the initiation and amplification of the clotting cascade. Assembly of the intrinsic pathway on the surface of the platelet also amplifies the clotting cascade.^{97,98} Finally, platelets bind plasma FV, which is internalized, processed, and stored as FVa.⁹⁹ This FVa is rapidly mobilized to the cell surface after platelet activation.

MPs.^{32,61,69,70,74–76} The contribution of these different cell types to the pool of circulating TF-positive MPs may depend on the underlying disease. Interestingly, several studies have shown that leukocyte-derived TF-positive MPs can bind to activated platelets through the interaction of PSGL-1 on the MPs with P-selectin expressed on the surface of activated platelets.^{77–80} This is an attractive model to explain how TF-positive MPs may be recruited to a thrombus and enhance its growth.

It should be noted that TF-negative MPs are also procoagulant.^{81,82} Therefore, the levels of functional TF in plasma cannot be quantified by simply measuring the procoagulant activity of isolated MPs. We have described an assay that selectively measures TF activity associated with captured MPs from a variety of cell types, including monocytes and endothelial cells.⁶⁹ However, one disadvantage of this assay is that it does not capture MPs generated by platelets, which may be a major source of TF-positive MPs under some conditions. Further studies are needed to determine the relative contribution of TF-positive and TF-negative MPs to thrombosis in different diseases.

Relative Contribution of Vessel Wall TF and MP TF to Arterial and Venous Thrombosis

Vessel wall TF and MP TF are likely to play different roles in arterial and venous thrombosis. Pharmacological inhibition

of TF has been shown to reduce both arterial and venous thrombosis in a variety of animal models. For instance, anti-TF antibodies, active-site inactivated FVIIa, tissue factor pathway inhibitor (TFPI), and small molecule inhibitors of the TF:FVIIa complex reduce thrombus size in arterial and venous models of thrombosis using rabbits and nonhuman primates.^{83–91} In addition, inhibition of the TF:FVIIa complex with recombinant nematode protein c2 reduced thrombin generation in patients undergoing elective coronary angioplasty.⁹²

Vascular smooth muscle cells in the arterial vessel wall express low levels of TF and a variety of cell types express TF in atherosclerotic lesions.⁹³ In a rabbit model, balloon catheter-induced endothelial denudation of the aorta or femoral artery increased TF expression in the vessel wall.^{86,94} Therefore, damage of normal or diseased arteries would expose TF to blood leading to the formation of an occlusive thrombus (Figure 3). In one study, disruption of the atherosclerotic plaques induced thrombosis, which was inhibited by active-site inhibited FVIIa.⁸⁶ Another study showed that arterial thrombosis was reduced by administration of TFPI.⁸⁷ We investigated the role of vessel wall TF in a mouse carotid arterial thrombosis model that involves acute oxidative damage of the vessel wall and denudation of the endothelium. We found that either reducing TF in all cells within the vessel

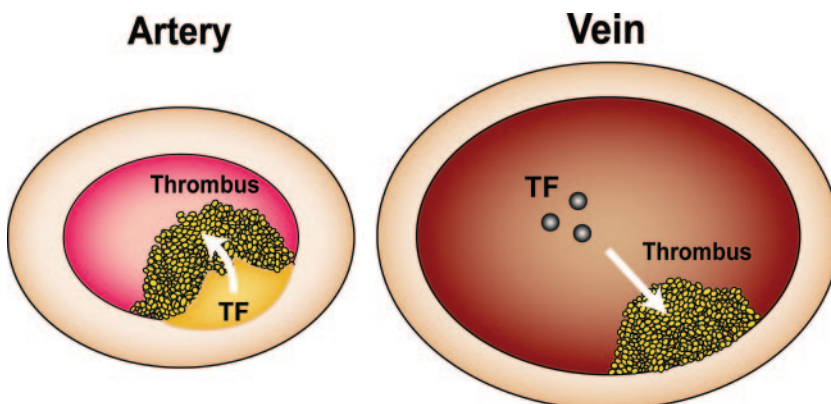


Figure 3. Contribution of vessel wall and MP TF to arterial and venous thrombosis. Arterial thrombosis, particularly after rupture of an atherosclerotic plaque, exposes large amounts of TF to blood and leads to the formation of an occlusive thrombus. The gold area in the arterial wall represents an atherosclerotic plaque. Venous thrombosis is not associated with disruption of the vessel wall. This suggests that MP TF plays a more important role than vessel wall TF in venous thrombosis.

wall or selectively reducing TF expression in vascular smooth muscle cells significantly prolonged the occlusion time⁹⁵ (Wang et al, in preparation). Therefore, inhibition of the TF:FVIIa complex may be an effective treatment strategy for the treatment of arterial thrombosis, particularly in atherosclerotic vessels.

Circulating TF is more likely to play a role in venous thrombosis that is not associated with vessel damage (Figure 3). One study showed that inhibition of TF reduced thrombus growth on a collagen-coated cotton thread inserted into the lumen of the jugular vein of a rabbit, suggesting that circulating TF contributed to thrombus growth in the uninjured vein.⁸³ We found that hematopoietic cell-derived TF-positive MPs contributed to the growth of a thrombus in laser injured microvasculature.⁹⁶ However, we did not find that hematopoietic cell-derived TF played a role in an inferior vena cava mouse model of venous thrombosis.⁹⁵ Further studies are required to determine the contribution of TF-positive MPs to venous thrombosis in animals with elevated levels of TF-positive MPs.

Summary

In conclusion, the extrinsic pathway of blood coagulation plays an essential role in hemostasis. Indeed, NovoSeven is used clinically to treat hemorrhages that arise from a variety of inherited and acquired conditions. Additional studies are required to elucidate the mechanism by which NovoSeven restores hemostasis. The role of platelet TF in hemostasis and thrombosis is currently unclear, and further studies are needed to determine whether or not this source of TF contributes to hemostasis and thrombosis. The presence of elevated levels of TF-positive MPs in blood may induce thrombosis associated with a variety of diseases and may represent a novel target for the antithrombotic drugs. The use of mice with cell type-specific deletion of the TF gene will also allow us to distinguish the roles of TF expression by different cell types in hemostasis and thrombosis.

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Disclosures

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