Brief Reviews

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.

See cover

TF and FVII in Hemostasis

Hemostasis is the protective physiological response to vascular injury that results in exposure of blood components to 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
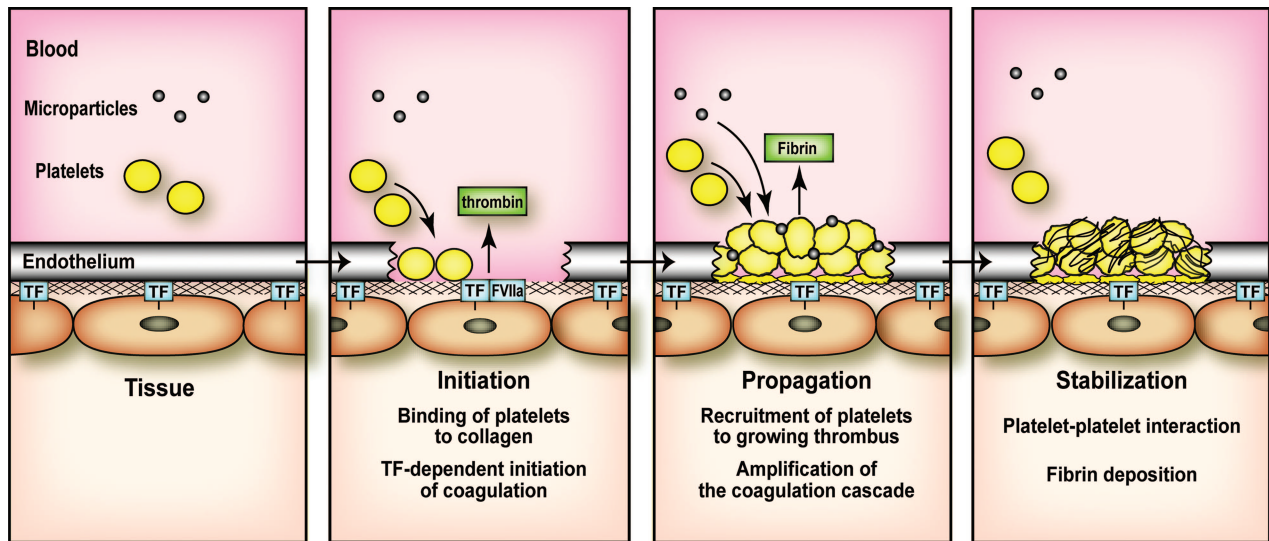
Figure 1. Formation of a clot at the site of blood vessel injury. In a healthy individual, TF expressed by vascular smooth muscle cells, pericytes, and adventitial fibroblasts in the vessel wall is physically separated from its ligand FVII/FVIIa by the endothelium. Vessel injury leads to the rapid binding 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 colleagues[28] 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 colleagues[29] 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 colleagues[46] 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 colleagues[59] 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 colleagues[62] found no detectable TF antigen or activity on quiescent or ionophore-stimulated platelets. Similarly, Osterud and colleagues[63] 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
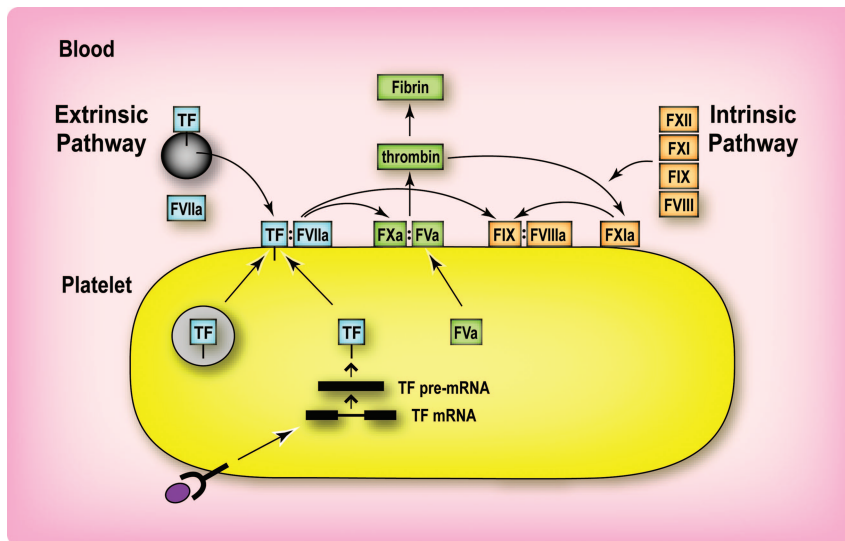
Figure 2. Potential role of platelets and the different coagulation pathways in the generation of thrombin and fibrin. Platelets have 3 sources of TF: MP TF, TF stored in α-granules, and de novo synthesized TF. These different sources participate in the initiation and amplification of the clotting cascade. 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
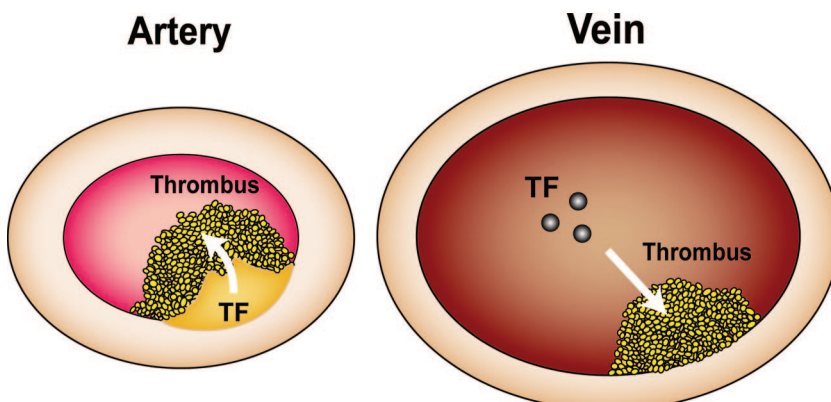

Figure 3. Contribution of vessel wall and MP TF to arterial and venous thrombosis. Arterial thrombosis, particularly after rupture of an atherosclerotic plaque, exposes large amounts of TF to blood and leads to the formation of an occlusive thrombus. The gold area in the arterial wall represents an atherosclerotic 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 time[95] (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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References

1. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol*. 1989;134:1087–1097.
2. Fleck RA, Rao LVM, Rapaport SI, Varki N. Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. *Thromb Res*. 1990;57:765–781.
3. Flössel C, Luther T, Müller M, Albrecht S, Kasper M. Immunohistochemical detection of tissue factor (TF) on paraffin sections of routinely fixed human tissue. *Histochemistry*. 1994;101:449–453.
4. Bouchard BA, Shatos MA, Tracy PB. Human brain pericytes differentially regulate expression of procoagulant enzyme complexes comprising the extrinsic pathway of blood coagulation. *Arterioscler Thromb Vasc Biol*. 1997;17:1–9.
5. Schecter AD, Spirn B, Rossikhina M, Giesen PL, Bogdanov V, Fallon JT, Fisher EA, Schnapp LM, Nemerson Y, Taubman MB. Release of active

6. tissue factor by human arterial smooth muscle cells. *Circ Res*. 2000;87: 126–132.
6. Mackman N, Sawdey MS, Keeton MR, Loskutoff DJ. Murine tissue factor gene expression *in vivo*: Tissue and cell specificity and regulation by lipopolysaccharide. *Am J Pathol*. 1993;143:76–84.
7. Erlich JH, Parry GCN, Fearns C, Muller M, Carmeliet P, Luther T, Mackman N. Tissue factor is required for uterine hemostasis and maintenance of the placental labyrinth during gestation. *Proc Natl Acad Sci U S A*. 1999;96:8138–8143.
8. Hartzell S, Ryder K, Lanahan A, Lau LF, Nathans D. A growth factor-responsive gene of murine BALB/c 3T3 cells encodes a protein homologous to human tissue factor. *Mol Cell Biol*. 1989;9:2567–2573.
9. Eddleston M, de la Torre JC, Oldstone MB, Loskutoff DJ, Edgington TS, Mackman N. Astrocytes are the primary source of tissue factor in the murine central nervous system. A role for astrocytes in cerebral hemostasis. *J Clin Invest*. 1993;92:349–358.
10. Tuddenham EGD, Pemberton S, Cooper DN. Inherited factor VII deficiency: genetics and molecular pathology. *Thromb Haemost*. 1995;74: 313–321.
11. Carmeliet P, Mackman N, Moons L, Luther T, Gressens P, Van Vlaenderen I, Demunck H, Kasper M, Breier G, Evrard P, Müller M, Risau W, Edgington T, Collen D. Role of tissue factor in embryonic blood vessel development. *Nature*. 1996;383:73–75.
12. Rosen ED, Chan JCY, Idusogie E, Clotman F, Vlasuk G, Luther T, Jalbert LR, Albrecht S, Zhong L, Lissens A, Schoonjans L, Moons L, Collen D, Castellino FJ, Carmeliet P. Mice lacking factor VII develop normally but suffer fatal perinatal bleeding. *Nature*. 1997;390:290–294.
13. Toomey JR, Kratzer KE, Lasky NM, Stanton JJ, Broze GJ Jr. Targeted disruption of the murine tissue factor gene results in embryonic lethality. *Blood*. 1996;88:1583–1587.
14. Bugge TH, Xiao Q, Kombrinck KW, Flick MJ, Holmback K, Danton MJS, Colbert MC, Witte DP, Fujikawa K, Davie EW, Degen JL. Fatal embryonic bleeding events in mice lacking tissue factor, the cell-associated initiator of blood coagulation. *Proc Natl Acad Sci U S A*. 1996;93:6258–6263.
15. Parry GCN, Erlich JH, Carmeliet P, Luther T, Mackman N. Low levels of tissue factor are compatible with development and hemostasis in mice. *J Clin Invest*. 1998;101:560–569.
16. Rosen ED, Xu H, Liang Z, Martin JA, Suckow M, Castellino FJ. Generation of genetically-altered mice producing very low levels of coagulation factorVII. *Thromb Haemost*. 2005;94:493–497.
17. Pawlinski R, Fernandes A, Kehrle B, Pedersen B, Parry G, Erlich J, Pyo R, Gutstein D, Zhang J, Castellino F, Melis E, Carmeliet P, Baretton G, Luther T, Taubman M, Rosen E, Mackman N. Tissue factor deficiency causes cardiac fibrosis and left ventricular dysfunction. *Proc Natl Acad Sci U S A*. 2002;99:15333–15338.
18. Pedersen B, Holscher T, Sato Y, Pawlinski R, Mackman N. A balance between tissue factor and tissue factor pathway inhibitor is required for embryonic development and hemostasis in adult mice. *Blood*. 2005;105: 1734–1741.
19. Pawlinski R, Pedersen B, Erlich J, Mackman N. Role of tissue factor in haemostasis, thrombosis, angiogenesis and inflammation: lessons from low tissue factor mice. *Thromb Haemost*. 2004;92:444–450.
20. Hedner U. NovoSeven as a universal haemostatic agent. *Blood Coagul Fibrinolysis*. 2000;11:S107–S111.
21. Abshire T, Kenet G. Recombinant factor VIIa: review of efficacy, dosing regimens and safety in patients with congenital and acquired factor VIII or IX inhibitors. *J Thromb Haemost*. 2004;2:899–909.
22. Margaritis P, Arruda VR, Aljamali M, Camire RM, Schlachterman A, High KA. Novel therapeutic approach for hemophilia using gene delivery of an engineered secreted activated Factor VII. *J Clin Invest*. 2004;113: 1025–1031.
23. Poon MC, d'Oiron R. Recombinant activated factor VII (NovoSeven) treatment of platelet-related bleeding disorders. International Registry on Recombinant Factor VIIa and Congenital Platelet Disorders Group. *Blood Coagul Fibrinolysis*. 2000;11 Suppl 1:S55–S68.
24. Mayer SA, Brun NC, Begtrup K, Broderick J, Davis S, Diringer MN, Skolnick BE, Steiner T. Recombinant activated factor VII for acute intracerebral hemorrhage. *N Engl J Med*. 2005;352:777–785.
25. Roberts HR. Recombinant factor VIIa: a general hemostatic agent Yes. *J Thromb Haemost*. 2004;2:1691–1694.
26. Levi M. Recombinant factor VIIa: a general hemostatic agent? Not yet. *J Thromb Haemost*. 2004;2:1695–1697.
27. Hoffman M, Monroe DM, Roberts HR. Human monocytes support factor X activation by factor VIIa, independent of tissue factor: Implications for

the therapeutic mechanism of high–dose factor VIIa in hemophilia. *Blood*. 1994;83:38–42.

28. Hoffman M, Monroe DM, Roberts HR. Platelet-dependent action of high-dose factor VIIa. *Blood*. 2002;100:364–365.

29. Butenas S, Brummel KE, Branda RF, Paradis SG, Mann KG. Mechanism of factor VIIa-dependent coagulation in hemophilia blood. *Blood*. 2002; 99:923–930.

30. Oliver JA, Monroe DM, Roberts HR, Hoffman M. Thrombin activates factor XI on activated platelets in the absence of factor XII. *Arterioscler Thromb Vasc Biol*. 1999;19:170–177.

31. Hoffman M, Monroe DM, III, Roberts HR Activated factor VII activates factors IX and X on the surface of activated platelets: thoughts on the mechanism of action of high-dose activated factor VII. *Blood Coagul Fibrinolysis*. 1998;9 Suppl 1::S61–S65.

32. Moosbauer C, Morgenstern E, Cuvelier SL, Manukyan D, Bidzhekov K, Albrecht S, Lohse P, Patel KD, Engelmann B. Eosinophils are a major intravascular location for tissue factor storage and exposure. *Blood*. 2007; 109:995–1002.

33. Taylor FB Jr, Chang A, Ruf W, Morrissey JH, Hinshaw L, Catlett R, Blick K, Edgington TS. Lethal *E. coli* septic shock is prevented by blocking tissue factor with monoclonal antibody. *Circ Shock*. 1991;33: 127–134.

34. Gregory SA, Morrissey JH, Edgington TS. Regulation of tissue factor gene expression in the monocyte procoagulant response to endotoxin. *Mol Cell Biol*. 1989;9:2752–2755.

35. Morrissey JH, Drake TA. Procoagulant response of the endothelium and monocytes. *In Pathophysiology of shock, sepsis and organ failure. Editors, Schlag, G. and Redl, H. Springer-Verlag*. 1993;564–574.

36. Franco RF, de Jonge E, Dekkers PEP, Timmerman JJ, Spek CA, van Deventer SJH, van Deursen P, van Kerkhoff L, van Gemen B, ten Cate H, van der Poll T, Reitsma PH. The in vivo kinetics of tissue factor messenger RNA expression during human endotoxemia: relationship with activation of coagulation. *Blood*. 2000;96:554–559.

37. de W, V, Hansen HR, Spronk HH, Timmerman JJ, Pannekoek H, Florquin S, Reitsma PH, ten Cate H. Differential expression of tissue factor mRNA and protein expression in murine sepsis. The role of the granulocyte revisited. *Thromb Haemost*. 2006;95:348–353.

38. Pawlinski R, Pedersen B, Schabbauer G, Tencati M, Holscher T, Boisvert W, Andrade-Gordon P, Frank RD, Mackman N. Role of tissue factor and protease activated receptors in a mouse model of endotoxemia. *Blood*. 2004;103:1342–1347.

39. Osterud B. Tissue factor in neutrophils: no. *J Thromb Haemost*. 2004;2: 218–220.

40. Nakamura S, Imamura T, Okamoto K. Tissue factor in neutrophils: yes. *J Thromb Haemost*. 2004;2:214–217.

41. Higure A, Okamoto K, Hirata K, Todoroki H, Nagafuchi Y, Takeda S, Katoh H, Itoh H, Ohsato K, Nakamura S. Macrophages and neutrophils infiltrating into the liver are responsible for tissue factor expression in a rabbit model of acute obstructive cholangitis. *Thromb Haemost*. 1996;75: 791–795.

42. Todoroki H, Nakamura S, Higure A, Okamoto K, Takeda S, Nagata N, Itoh H, Ohsato K. Neutrophils express tissue factor in a monkey model of sepsis. *Surgery*. 2000;127:209–216.

43. Maugeri N, Brambilla M, Camera M, Carbone A, Tremoli E, Donati MB, De Gaetano G, Cerletti C. Human polymorphomuclear leukocytes produce and express functional tissue factor upon stimulation. *J Thromb Haemost*. 2006;4:1323–1330.

44. Ritis K, Doumas M, Mastellos D, Micheli A, Giaglis S, Magotti P, Rafail S, Kartalis G, Sideras P, Lambris JD. A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. *J Immunol*. 2006;177:4794–4802.

45. Parry GCN, Mackman N. Transcriptional regulation of tissue factor expression in human endothelial cells. *Arterioscler Thromb*. 1995;15: 612–621.

46. Drake TA, Taylor FB Jr. Immunohistochemical assessment of tissue factor and thrombomodulin expression in tissues of baboons with lethal *e. coli* sepsis. *FASEB J*. 1991;5:A1437.

47. Lupu C, Westmuckett AD, Peer G, Ivanciu L, Zhu H, Taylor FB Jr, Lupu F. Tissue Factor-Dependent Coagulation Is Preferentially Up-Regulated within Arterial Branching Areas in a Baboon Model of Escherichia coli Sepsis. *Am J Pathol*. 2005;167:1161–1172.

48. Hara M, Ono K, Hwang MW, Iwasaki A, Okada M, Nakatani K, Sasayama S, Matsumori A. Evidence for a role of mast cells in the evolution to congestive heart failure. *J Exp Med*. 2002;195:375–381.

49. Solovey A, Gui LH, Key NS, Hebbel RP. Tissue factor expression by endothelial cells in sickle cell anemia. *J Clin Invest*. 1998;101: 1899–1904.

50. Solovey A, Lollander R, Shet A, Milbauer LC, Choong S, Panoskaltsis-Mortari A, Blazar BR, Kelm RJ, Hebbel RP. Endothelial Cell Expression of Tissue Factor in Sickle Mice is Augmented by Hypoxia/Reoxygenation and Inhibited by Lovastatin. *Blood*. 2004;104:840–846.

51. Contrino J, Hair G, Kreutzer DL, Rickles FR. In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. *Nature Med*. 1996;2:209–215.

52. Shoji M, Hancock WW, Abe K, Micko C, Casper KA, Baine RM, Wilcox JN, Danave I, Dillehay DL, Matthews E, Contrino J, Morrissey JH, Gordon S, Edgington TS, Kudryk B, Kreutzer DL, Rickles FR. Activation of coagulation and angiogenesis in cancer. Immunohistochemical localization *in situ* of clotting proteins and vascular endothelial growth factor in human cancer. *Am J Pathol*. 1998;152:399–411.

53. Luther T, Flossel C, Albrecht S, Kotzsch M, Muller M. Tissue factor expression in normal and abnormal mammary gland. *Nat Med*. 1996;2: 491–492.

54. Thiruvikraman SV, Guha A, Roboz J, Taubman MB, Nemerson Y, Fallon JT In situ localization of tissue factor in human atherosclerotic plaques by binding of digoxigenin-labeled factors VIIa and X. *Lab Invest*. 1996; 75:451

55. Lang IM, Mackman N, Kriett JM, Moser KM, Schleef RR. Prothrombotic activation of pulmonary arterial endothelial cells in a patient with tuberculosis. *Hum Pathol*. 1996;27:423–427.

56. More L, Sim R, Hudson M, Dhillon AP, Pounder R, Wakefield AJ. Immunohistochemical study of tissue factor expression in normal intestine and idiopathic inflammatory bowel disease. *J Clin Pathol*. 1993; 46:703–708.

57. Muller D, Mervaala E, Dechend R, Fiebeler A, Park J-K, Schmidt F, Theuer J, Breu V, Mackman N, Luther T, Schneider W, Gulba D, Ganten D, Haller H, Luft F. Angiotensin II (AT$_1$) receptor blockade reduces vascular tissue factor in angiotensin II-induced cardiac vasculopathy. *Am J Pathol*. 2000;157:111–122.

58. Hölschermann H, Bohle RM, Zeller H, Schmidt H, Stahl U, Fink L, Grimm H, Tillmanns H, Haberbosch W. In situ detection of tissue factor within the coronary intima in rat cardiac allograft vasculopathy. *Am J Pathol*. 1999; 154:211–220.

59. Zillmann A, Luther T, Muller I, Kotzsch M, Spannagl M, Kauke T, Oelschlagel U, Zahler S, Engelmann B. Platelet-associated tissue factor contributes to the collagen-triggered activation of blood coagulation. *Biochem Biophys Res Commun*. 2001;281:603–609.

60. Muller I, Klocke A, Alex M, Kotzsch M, Luther T, Morgenstern E, Zieseniss S, Zahler S, Preissner K, Engelmann B. Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. *FASEB J*. 2003;17:476–478.

61. Siddiqui FA, Desai H, Amirkhosravi A, Amaya M, Francis JL. The presence and release of tissue factor from human platelets. *Platelets*. 2002;13:247–253.

62. Butenas S, Bouchard BA, Brummel-Ziedins KE, Parhami-Seren B, Mann KG. Tissue factor activity in whole blood. *Blood*. 2005;105:2764–2770.

63. Osterud B, Bjorklid E. Sources of tissue factor. *Semin Thromb Hemost*. 2006;32:11–23.

64. Schwertz H, Tolley ND, Foulks JM, Denis MM, Risenmay BW, Buerke M, Tilley RE, Rondina MT, Harris EM, Kraiss LW, Mackman N, Zimmerman GA, Weyrich AS. Signal-dependent splicing of tissue factor pre-mRNA modulates the thrombogenicity of human platelets. *J Exp Med*. 2006;203:2433–2440.

65. Camera M, Frigerio M, Toschi V, Brambilla M, Rossi F, Cottell DC, Maderna P, Parolari A, Bonzi R, De Vincenti O, Tremoli E. Platelet activation induces cell-surface immunoreactive tissue factor expression, which is modulated differently by antiplatelet drugs. *Arterioscler Thromb Vasc Biol*. 2003;23:1690–1696.

66. Panes O, Matus V, Saez CG, Quiroga T, Pereira J, Mezzano D Human platelets synthesize and express functional tissue factor. *Blood*. In press.

67. Misumi K, Ogawa H, Yasue H, Soejima H, Suefuji H, Nishiyama K, Takazoe K, Kugiyama K, Tsuji I, Kumeda K, Nakamura S. Comparison of plasma tissue factor levels in unstable and stable angina pectoris. *Am J Cardiol*. 1998;81:22–26.

68. Morel O, Toti F, Hugel B, Bakouboula B, Camoin-Jau L, Dignat-George F, Freyssinet JM. Procoagulant microparticles: disrupting the vascular homeostasis equation? *Arterioscler Thromb Vasc Biol*. 2006;26: 2594–2604.

69. Aras O, Shet A, Bach RR, Hysjulien JL, Slungaard A, Hebbel RP, Escolar G, Jilma B, Key NS. Induction of microparticle- and cell-associated intravascular tissue factor in human endotoxemia. *Blood*. 2004;103: 4545–4553.

70. Shet AS, Aras O, Gupta K, Hass MJ, Rausch DJ, Saba N, Koopmeiners L, Key NS, Hebbel RP. Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. *Blood*. 2003;102:2678–2683.

71. Tesselaar ME, Romijn FP, van dL, I, Prins FA, Bertina RM, Osanto S Microparticle-associated tissue factor activity: a link between cancer and thrombosis *J Thromb Haemost*. In press.

72. Rauch U, Antoniak S. Tissue factor-positive microparticles in blood associated with coagulopathy in cancer. *Thromb Haemost*. 2007;97:9–10.

73. Nieuwland R, Berckmans RJ, McGregor S, Boing AN, Romijn FP, Westendorp RG, Hack CE, Sturk A. Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood*. 2000;95: 930–935.

74. Nieuwland R, Berckmans RJ, Rotteveel-Eijkman RC, Maquelin KN, Roozendaal KJ, Jansen PGM, ten Have K, Eijsman L, Hack CE, Sturk A. Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circulation*. 1997;96:3534–3541.

75. Leroyer AS, Isobe H, Leseche G, Castier Y, Wassef M, Mallat Z, Binder BR, Tedgui A, Boulanger CM. Cellular origins and thrombogenic activity of microparticles isolated from human atherosclerotic plaques. *J Am Coll Cardiol*. 2007;49:772–777.

76. Hron G, Kollars M, Weber H, Sagaster V, Quehenberger P, Eichinger S, Kyrle PA, Weltermann A. Tissue factor-positive microparticles: cellular origin and association with coagulation activation in patients with colo-rectal cancer. *Thromb Haemost*. 2007;97:119–123.

77. Furie B, Furie BC. Thrombus formation in vivo. *J Clin Invest*. 2005;115: 3355–3362.

78. Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coag-ulation triad. *J Thromb Haemost*. 2005;3:1590–1596.

79. Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated plate-lets to initiate coagulation. *Blood*. 2005;106:1604–1611.

80. Rauch U, Bonderman D, Bohrmann B, Badimon JJ, Himber J, Riederer MA, Nemerson Y. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. *Blood*. 2000;96:170–175.

81. Sturk-Maquelin KN, Nieuwland R, Romijn FP, Eijsman L, Hack CE, Sturk A. Pro- and non-coagulant forms of non-cell-bound tissue factor in vivo. *J Thromb Haemost*. 2003;1:1920–1926.

82. Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Cam-phausen RT, Widom A, Xia L, Kazazian HH Jr, Schaub RG, McEver RP, Wagner DD. Interaction of P-selectin and PSGL-1 generates micropar-ticles that correct hemostasis in a mouse model of hemophilia A. *Nat Med*. 2003;9:1020–1025.

83. Himber J, Wohlgensinger C, Roux S, Damico LA, Fallon JT, Kirchhofer D, Nemerson Y, Riederer MA. Inhibition of tissue factor limits the growth of venous thrombus in the rabbit. *J Thromb Haemost*. 2003;1: 889–895.

84. Arnljots B, Ezban M, Hedner U. Prevention of experimental arterial thrombosis by topical administration of active site-inactivated factor VIIa. *J Vasc Surg*. 1997;25:341–346.

85. Young WB, Mordenti J, Torkelson S, Shrader WD, Kolesnikov A, Rai R, Liu L, Hu H, Leahy EM, Green MJ, Sprengeler PA, Katz BA, Yu C, Janc JW, Elrod KC, Marzec UM, Hanson SR. Factor VIIa inhibitors: chemical optimization, preclinical pharmacokinetics, pharmacodynamics, and efficacy in an arterial baboon thrombosis model. *Bioorg Med Chem Lett*. 2006;16:2037–2041.

86. Chi L, Gibson G, Peng YW, Bousley R, Brammer D, Rekhter M, Chen J, Leadley R. Characterization of a tissue factor/factor VIIa-dependent model of thrombosis in hypercholesterolemic rabbits. *J Thromb Haemost*. 2004;2:85–92.

87. Asada Y, Hara S, Tsuneyoshi A, Hatakeyama K, Kisanuki A, Marutsuka K, Sato Y, Kamikubo Y, Sumiyoshi A. Fibrin-rich and platelet-rich thrombus formation on neointima: recombinant tissue factor pathway inhibitor prevents fibrin formation and neointimal development following repeated balloon injury of rabbit aorta. *Thromb Haemost*. 1998;80: 506–511.

88. Roque M, Reis ED, Fuster V, Padurean A, Fallon JT, Taubman MB, Chesebro JH, Badimon JJ. Inhibition of tissue factor reduces thrombus formation and intimal hyperplasia after porcine coronary angioplasty. *J Am Coll Cardiol*. 2000;36:2303–2310.

89. Suleymanov OD, Szalony JA, Salyers AK, LaChance RM, Parlow JJ, South MS, Wood RS, Nicholson NS. Pharmacological interruption of acute thrombus formation with minimal hemorrhagic complications by a small molecule tissue factor/factor VIIa inhibitor: comparison to factor Xa and thrombin inhibition in a nonhuman primate thrombosis model. *J Pharmacol Exp Ther*. 2003;306:1115–1121.

90. Olivero AG, Eigenbrot C, Goldsmith R, Robarge K, Artis DR, Flygare J, Rawson T, Sutherlin DP, Kadkhodayan S, Beresini M, Elliott LO, DeGuzman GG, Banner DW, Ultsch M, Marzec U, Hanson SR, Refino C, Bunting S, Kirchhofer D. A selective, slow binding inhibitor of factor VIIa binds to a nonstandard active site conformation and attenuates thrombus formation in vivo. *J Biol Chem*. 2005;280:9160–9169.

91. Szalony JA, Suleymanov OD, Salyers AK, Panzer-Knodle SG, Blom JD, LaChance RM, Case BL, Parlow JJ, South MS, Wood RS, Nicholson NS. Administration of a small molecule tissue factor/factor VIIa inhibitor in a non-human primate thrombosis model of venous thrombosis: effects on thrombus formation and bleeding time. *Thromb Res*. 2003;112:167–174.

92. Moons AH, Peters RJ, Bijsterveld NR, Piek JJ, Prins MH, Vlasuk GP, Rote WE, Buller HR. Recombinant nematode anticoagulant protein c2, an inhibitor of the tissue factor/factor VIIa complex, in patients undergoing elective coronary angioplasty. *J Am Coll Cardiol*. 2003;41:2147–2153.

93. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci U S A*. 1989;86:2839–2843.

94. Hatakeyama K, Asada Y, Marutsuka K, Kataoka H, Sato Y, Sumiyoshi A. Expression of tissue factor in the rabbit aorta after balloon injury. *Atherosclerosis*. 1998;139:265–271.

95. Day SM, Reeve JL, Pedersen B, Farris DM, Myers DD, Im M, Wakefield TW, Mackman N, Fay WP. Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood*. 2005;105: 192–198.

96. Chou J, Mackman N, Merrill-Skoloff G, Pedersen B, Furie BC, Furie B. Hematopoietic Cell-Derived Microparticle Tissue Factor Contributes to Fibrin Formation During Thrombus Propagation. *Blood*. 2004;104: 3190–3197.

97. Walsh PN. Roles of factor XI, platelets and tissue factor-initiated blood coagulation. *J Thromb Haemost*. 2003;1:2081–2086.

98. Ahmad SS, London FS, Walsh PN. Binding studies of the enzyme (factor IXa) with the cofactor (factor VIIIa) in the assembly of factor-X acti-vating complex on the activated platelet surface. *J Thromb Haemost*. 2003;1:2348–2355.

99. Gould WR, Simioni P, Silveira JR, Tormene D, Kalafatis M, Tracy PB. Megakaryocytes endocytose and subsequently modify human factor V in vivo to form the entire pool of a unique platelet-derived cofactor. *J Thromb Haemost*. 2005;3:450–456.