

Evolving role of tissue factor and its pathway inhibitor

Sagar N. Doshi, BSc, MBChB, MRCP; Jonathan D. Marmur, MD, FRCP(c), FACC

Objective: To review the experimental and clinical evidence of the emerging role of tissue factor in intravascular thrombosis and to examine evidence supporting the potential use of tissue factor pathway inhibitor as an antithrombotic therapeutic agent.

Data Sources and Study Selection: A PubMed search was conducted encompassing articles in the English language relating to tissue factor and tissue factor pathway inhibitor in intravascular coagulation.

Conclusions: Tissue factor, a membrane-bound procoagulant glycoprotein, is the initiator of the extrinsic clotting cascade, which is the predominant coagulation pathway *in vivo*. The traditional view localizes tissue factor to extravascular sites, where it remains sequestered from circulating factor VII until vascular integrity is disrupted or until tissue factor expression is induced in endothelial cells or monocytes. This perspective has been challenged since the discovery of tissue factor antigen in plasma, on circulating microparticles, and on leukocytes in whole blood. Recently, the apparent role of tissue factor has expanded with the

demonstration that this molecule also functions as a signaling receptor. Recombinant tissue factor pathway inhibitor, an analogue of the physiologic inhibitor of tissue factor, is a potent inhibitor of thrombus formation in experimental models. In summary, the tissue factor pathway initiates thrombosis *in vivo*. In addition to its classic tissue-bound distribution, recently discovered blood-borne tissue factor may have an important procoagulant function. Despite showing promise in early human studies, a recently completed phase 3 trial of recombinant tissue factor pathway inhibitor in severe sepsis failed to show a reduction in the primary end point of 28-day all-cause mortality. Tissue factor pathway inhibitor, however, remains a plausible therapeutic agent in other conditions of increased thrombogenicity, such as acute coronary syndromes, and further studies to examine this potential are warranted. (Crit Care Med 2002; 30[Suppl.]:S241–S250)

KEY WORDS: atherosclerosis; sepsis; tissue factor; tissue factor pathway inhibitor

Tissue factor (TF), a transmembrane procoagulant glycoprotein and member of the cytokine receptor superfamily, triggers the extrinsic coagulation cascade, the pathway considered to be responsible for the initiation of hemostasis and thrombosis *in vivo* (1, 2). Recently, the apparent role of TF expanded with the demonstration that this protein also functions as a signaling receptor (3). The TF molecule consists of three distinct sections: a short cytoplasmic domain (19 residues), a transmembrane domain (23 residues), and a large extracellular domain (219 residues). The extracellular domain binds with factor VII/VIIa in circulating blood to form a catalytic complex that converts inactive factors IX and X to their active forms, IXa and Xa, with the subsequent formation of thrombin and deposition of fibrin (Fig. 1) (4–6).

A number of observations in both human and nonhuman primates suggest that activation of the coagulation cascade

in vivo is initiated via the TF pathway. Patients with intrinsic pathway coagulation factor deficiencies, such as factor XI, have normal basal factor IXa generation, whereas patients lacking extrinsic pathway factors, such as factor VII, have depressed factor IX activation (6). Infusion of recombinant factor VIIa in patients with hereditary factor VII deficiency results in thrombin generation, whereas infusion of factors IX or VIII in patients deficient in these factors does not result in thrombin generation (7). In addition, generation of thrombin by recombinant factor VIIa is abolished in chimpanzees by pretreatment with a monoclonal anti-TF antibody, suggesting that activation of coagulation by factor VIIa requires association with TF (4). These observations point to the predominant role that TF and the extrinsic cascade play in maintaining the basal procoagulant tone of the organism. What, then, is the role of the intrinsic cascade? In the revised theory of coagulation, TF is responsible for initiation of coagulation; however, as a result of inhibition by TF pathway inhibitor (TFPI), the procoagulant activity of TF requires amplification by the intrinsic pathway (8). Traditionally, activation of the intrinsic cascade has been understood

to involve exposure of blood to a negatively charged surface, resulting in a sequence of cleavage reactions culminating in the conversion of factor IX to its active form IXa. However, a landmark observation in 1990 (6) established that the TF–factor VIIa complex is capable of mediating the conversion of factor IX to IXa directly, thereby establishing a link between the two pathways. Thus, TF seems to have a dual role with respect to coagulation: 1) TF initiates coagulation through activation of factor X, and 2) through its action on factor IX, TF supports amplification. An additional mechanism that supports the importance of the intrinsic cascade is activation of factor VIII through feedback mechanisms involving thrombin (see Fig. 1).

The procoagulant role of TF seems to be vital for development. TF deficiency in mice is lethal, with death occurring *in utero* from hemorrhage (9). Further work revealed that insertion of the human TF minigene, which results in approximately 1% of normal TF activity, rescued TF-deficient mice, indicating that low levels of TF expression are compatible with survival (10). Furthermore, this rescue was dependent on the extracellular domain of TF. Insertion of a TF

From the Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

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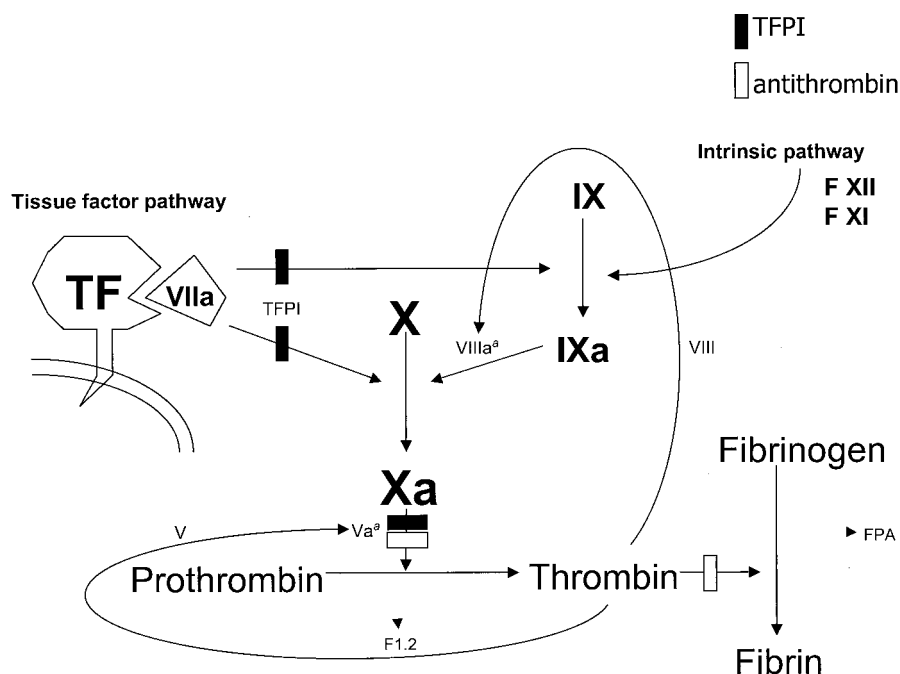


Figure 1. Schema of the coagulation mechanism. Although the coagulation cascade is classically depicted as initiated via either the intrinsic or extrinsic (TF) pathway, several lines of evidence point to the TF pathway as the primary trigger of clotting *in vivo*. Both pathways converge at the conversion of factor X to Xa, which yields thrombin, the central bioregulatory molecule of thrombosis. Thrombin promotes its own generation through positive feedback mechanisms involving activation of factors V and VIII, which are inhibited by activated protein C. Two additional endogenous inhibitory mechanisms include antithrombin (which binds unfractionated and low molecular weight heparin, and pentasaccharide, to inhibit factors IIa and Xa) and tissue factor pathway inhibitor (TFPI, which blocks both the generation and activity of factor Xa). In the revised theory of coagulation, the rapid inactivation of factor Xa by TFPI is counteracted by the activity of the intrinsic cascade, which promotes further Xa generation through factor IXa. Thus, the role of the intrinsic pathway is to sustain and accelerate the thrombotic reactions set into motion initially via the extrinsic cascade. Generation of factor IXa may occur via the intrinsic cascade and via the TF-factor VIIa complex, thereby establishing a role for TF in both pathways. Prothrombin fragment F1.2 and fibrinopeptide A (FPA) reflect thrombin generation and activity, respectively. ^aInactivated by activated protein C; protein C is activated when thrombin binds thrombomodulin.

minigene lacking the cytoplasmic domain achieved rescue, whereas a TF minigene lacking the extracellular domain was incapable of such rescue (11).

TF ORIGIN AND DISTRIBUTION

Under appropriate conditions, a wide variety of cells are able to synthesize and express TF. In extravascular tissues, monocytes, macrophages and fibroblasts have been found to express TF constitutively (12–14). In a distribution consistent with its primary function as an initiator of hemostasis, TF is localized in an envelope-like distribution with high levels in the adventitia of blood vessels, the fibrous capsules of organs, and the epithelium of the skin and internal mucosae—sequestered from circulating factor VII but poised to activate coagulation on disruption of the vessel wall (12). The

absence of significant levels of active TF in the endothelium and peripheral blood cells seemed to lend further support to the concept that maintenance of a physical barrier between TF and blood is necessary for the normal regulation of coagulation and thrombosis (12–14). However, this classic view has recently been challenged with the discovery of blood-borne TF (*vide infra*).

In addition to its constitutive expression, TF antigen can be induced in various cell types after exposure to a wide variety of stimuli. In culture, endotoxins, tumor necrosis factor- α , phorbol esters, growth factors, and lipoproteins stimulate the expression of TF on endothelial cells (15–19), smooth muscle cells (SMCs) (20–22), and blood monocytes (23–25). Numerous studies support the emerging role of CD40–CD40-ligand in-

teractions in atherosclerosis, thrombosis, and inflammation (26), and recently, the binding of CD40-ligand by vascular SMCs *in vitro* was shown to induce TF expression (27). C-reactive protein, an acute phase protein, has also been shown to induce TF expression by human monocytes, although C-reactive protein was incapable of inducing TF in human umbilical vein endothelial cells (28). *In vitro*, inducible TF activity on monocytes is reduced by cerivastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (29). The TF-lowering action of statins may be explained by their reduction of lipoproteins, although an alternative mechanism may be via reduction of C-reactive protein (30).

TF IN DISEASE STATES

Atherosclerosis. In the normal arterial vasculature of animals and humans, TF is localized to cells in the adventitia with only trace quantities detectable in the media or intima and no detectable endothelial TF (12, 13, 31). TF messenger RNA in rat aorta SMCs can be rapidly induced after balloon injury, providing a procoagulant that may result in thrombus initiation or propagation (31). In atherosclerotic plaques from human carotid arteries, aortas, and coronary arteries, heavy deposition of TF antigen has been found in macrophage foam cells and within the cell-free necrotic core (14, 32–34). Acute coronary syndromes are believed to be the consequence of spontaneous fissuring of atheromatous plaques, with subsequent thrombus formation (35–37). TF in atherosclerotic plaques has been shown to be active and capable of generating activated factor X (33, 34). The thrombogenicity of atherosclerotic plaques of varying composition has been studied, with the finding that platelet deposition and TF staining are greatest in lipid-rich plaques, indicating that TF may be an important determinant of the thrombogenicity of atheromatous lesions (32). Further evidence of the functional significance of TF found in atherosclerotic plaques comes from the observation that the quantity of active TF correlates with the degree of intracoronary thrombin generation *in vivo* (38). Determination of TF expression using anti-TF antibodies in coronary atherectomy specimens from patients with angina has revealed that TF concentration (39, 40) and activity (34) are greater in patients with unstable angina compared with those

with stable angina. TF localization within plaques also seems to vary according to the clinical syndrome. In stable angina, TF seems to be mainly located in the acellular component, whereas in unstable angina, it is found predominantly in macrophages and SMCs. Furthermore, TF in the acellular lipid core is highly correlated with the presence of macrophages, suggesting that macrophages contribute not only to the bulk of the lipid core but also to its thrombogenicity (40).

The origin of TF in the acellular lipid-rich core is incompletely understood (41). Apoptosis is an important step in the development and progression of atherosclerotic plaques (42), and apoptotic cells and microparticles (MPs) have been shown to possess high procoagulant activity (43, 44). Recently, Mallat et al. (45) localized the majority of TF expression in human atherosclerotic plaques to areas of high density of apoptotic cells and debris. Furthermore, TF activity in the acellular lipid core was found mainly on apoptotic MPs of monocytic and lymphocytic origin. *In vitro*, SMCs have been shown to be capable of releasing TF-positive MPs, suggesting that SMCs in atherosclerotic plaques may be a further source of TF (23). Infection with *Chlamydia pneumoniae* is believed to promote atherosclerosis and to precipitate acute coronary syndromes, although the precise mechanisms are incompletely understood. Evidence that infection of human endothelial cells with *C. pneumoniae* results in a marked increase in TF expression, along with increased expression of interleukin (IL)-6 and plasminogen activator inhibitor-1 (46, 47), may partly explain the disease.

Sepsis. In human and animal models of sepsis, TF levels are elevated and it is believed that this directly contributes to the pathophysiology that leads to multiple organ failure. Evidence for the importance of TF comes from experimental observations that specific anti-TF antibodies significantly inhibit the coagulopathy that follows induced sepsis in primate models (48, 49). However, the origin of TF in sepsis is unclear. Bacterial endotoxins induce the production of a number of proinflammatory cytokines, including tumor necrosis factor- α , IL-1, IL-6, and IL-8. During infection and after stimulation with cytokines and endotoxins, TF is rapidly expressed on the surface of circulating monocytes and endothelial cells (50–55). However, monocytes are the only cells found in the peripheral blood

capable of expressing TF (56), and the contribution and functional significance of endothelium-derived TF in sepsis remains uncertain.

Recently, increased levels of procoagulant MPs have been discovered in patients with meningococcal sepsis compared with healthy controls. Using flow cytometry, investigators studied the cellular origin of the MPs and found that they originated from a variety of cells, including platelets, monocytes, endothelial cells, and granulocytes. Furthermore, these MPs stained positive for annexin V, indicating the presence of phosphatidylserine, an essential cofactor for TF activity (57).

CIRCULATING TF: A CHALLENGE TO PREVIOUS PARADIGMS

Until recently the conventional view of activation of the TF pathway was that arterial thrombosis after vascular injury was the result of exposure of subendothelial TF to factor VII/VIIa and platelets. This dogma has recently been challenged after observations that TF antigen and activity can be isolated from whole blood and plasma in disease states and from healthy volunteers (58–66).

Giesen et al. (60) elegantly demonstrated formation of active TF-staining thrombus on pig arterial media and collagen-coated glass slides exposed to freshly drawn and flowing human blood in a laminar flow chamber (Fig. 2). Both substrates were initially devoid of TF staining, indicating that the TF had originated from blood. Furthermore, addition of TF inhibitors abolished thrombus formation. TF activity was found in both extracted plasma and whole blood of the healthy subjects. By immunostaining, TF expression was identified in neutrophils and monocytes, suggesting that these cells may be a source of plasma TF.

MPs and TF in Plasma. Activated eukaryotic cells shed fragments of their plasma membrane into the extracellular space (67). Human umbilical vein endothelial cells stimulated with tumor necrosis factor- α , for example, have been shown to release MPs detectable by electron microscopy (Fig. 3) (68). These fragments arise from an exocytotic budding process. MPs include cytoplasmic components and membrane elements such as phospholipids and cell-surface receptors. These MPs possess procoagulant activity because of the presence of TF and can be

detected at low levels in healthy volunteers, with elevated levels in prothrombotic states such as acute coronary syndromes (69), paroxysmal nocturnal hemoglobinuria (70), antiphospholipid syndrome (70), during activated coagulation (71), and in sepsis (57). *In vitro* and *in vivo*, MPs have been shown to originate from a wide variety of cell types, including monocytes (45, 72), endothelial cells (73, 74), platelets (57), polymorphonuclear cells (75), lymphocytes (45), and SMCs (21). Whether all of these cell types give rise to MPs in disease states is uncertain, but recent research found MPs in blood from all these cell types in patients with meningococcal sepsis (57). Recently, circulating MPs extracted from patients presenting with an acute myocardial infarction were demonstrated to impair endothelium-dependent relaxation in rat aortic rings, indicating reduced bioavailability of nitric oxide. In contrast, MPs obtained from nonischemic controls had no effect on rat aortic rings (47). The explanation for this differential effect is obscure but may be caused by different cellular origins or changes in proteins or phospholipid composition of the MPs (69).

MPs generated *in vivo* have been demonstrated to be capable of initiating coagulation. Pericardial plasma taken from patients during cardiopulmonary bypass was found to be rich in platelet-derived MPs and highly procoagulant. This activity was lost after removal of its MPs by high-speed centrifugation, whereas the corresponding MP pellet was strongly procoagulant (75). The functional importance of MPs in human disease states has not been well defined. The possibility that MPs may be involved in cellular signaling has recently been raised (76), and the mechanisms of cellular activation by platelet MPs have been reviewed elsewhere (77).

SIGNIFICANCE OF CIRCULATING TF

It has been proposed that TF in the vessel wall alone is insufficient for the rapid propagation of luminal thrombus; it has been calculated that proteins the size of TF would take several hours to diffuse to the apex of a thrombus 1 mm in height (Fig. 4) (63, 78). Thus, TF present in the blood compartment is likely to contribute not only to thrombus initiation but also to its propagation.

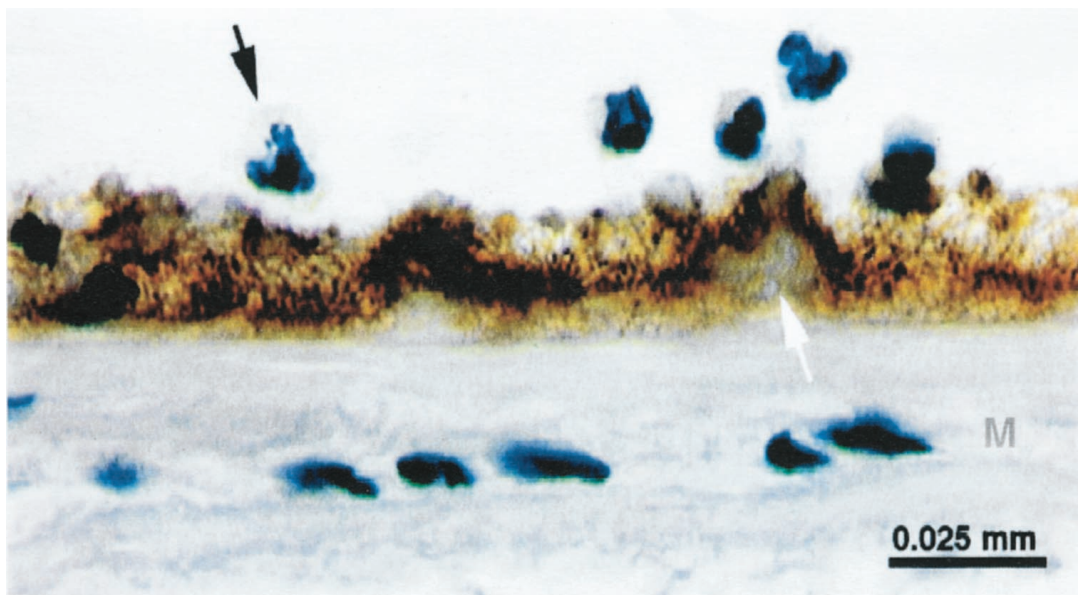


Figure 2. Tissue factor (TF) staining (brown) of a thrombus formed on pig aortic media (M) after passage of freshly drawn, flowing human blood. TF staining was absent in the media, indicating that the TF originated from blood. TF staining was confined to the thrombus and some of the neutrophils (black arrow). Much of the immunostained TF (pAb-TF) appears to be extracellular and associated with fibrin (white arrow). Reproduced with permission from Giesen et al (60).

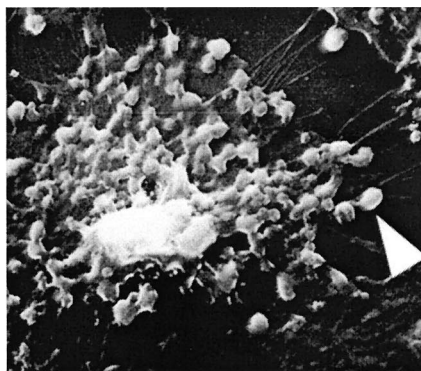
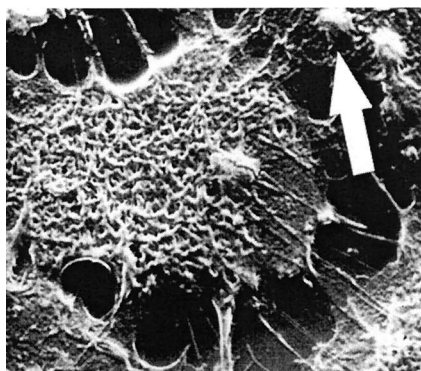


Figure 3. Scanning electron micrographs (original magnification, $\times 3000$) of first-passage resting human umbilical vein endothelial cells (HUVECs) (left) and after tumor necrosis factor- α (TNF- α) stimulation (right). Left, arrow shows surface blebs on resting HUVECs. Right, TNF- α -stimulated HUVECs release microparticles (arrowhead). Reproduced with permission from Combes V, Simon AC, Grau GE, et al: In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. *J Clin Invest* 1999; 104:93–102.

Elevated levels of TF antigen in blood have been detected in patients with coronary heart disease and hypercholesterolemia compared with healthy controls (59, 61). Furthermore, levels of circulating TF have been found to be increased in unstable compared with stable coronary heart disease (65, 79). Consistent with these findings, increased levels of TF antigen have been discovered in hematologic disorders associated with heightened intravascular coagulation, including sickle cell anemia (66), antiphospholipid syndrome (80), and disseminated intravascular coagulation (58, 81). Collec-

tively, these observations support the hypothesis that TF is associated with increased thrombogenicity in these disease states.

DE-ENCRYPTION

Virtually all TF on cell membranes in blood is encrypted and inactive. Encrypted TF is capable of binding factor VIIa, but the complex remains catalytically inactive and incapable of initiating coagulation (82, 83). It seems probable that encryption serves to prevent unwanted activation of intravascular throm-

bosis. Precisely how TF is encrypted is incompletely understood. One suggested mechanism is the localization of TF in caveolae—flask-shaped microinvaginations of the cell membrane. In one study, TF on human SMCs was found within caveolae (Fig. 5) (84). This mechanism may perhaps underlie the observation by Schecter et al. (23) that TF activity in smooth muscle increases after cell lysis. After blockade of surface TF by antibodies and cell lysis, they found that one third of TF is intracellular and two thirds is present on the cell surface. These intracellular stores of TF may become available for surface expression during coagulation. Phosphatidylserine, a negatively charged membrane phospholipid, is an essential cofactor for TF activation, and increased availability or altered distribution may further regulate TF de-encryption.

A potential mechanism of de-encryption is via an increase in intracellular calcium, which has been shown to increase TF activity in a dose-dependent fashion without alteration of TF expression, independent of the intracellular domain and phosphatidylserine (85, 86). Thus, TF procoagulant activity may be triggered by a Ca^{2+} influx into the cytosol (87). *In vitro*, the transfer of encrypted TF from a monocyte-derived cell line (THP-1) to activated platelets in thrombi has been shown to be dependent on in-

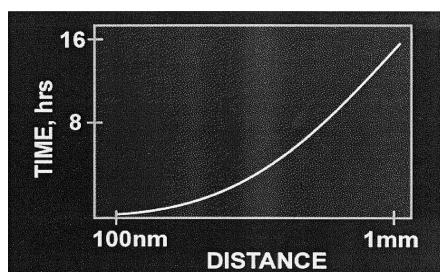


Figure 4. Graph showing hypothetical diffusion time for tissue factor (TF) to travel a distance of 1 mm. The classic view of thrombosis localizes TF to extravascular sites. After vessel wall injury and thrombus formation on the luminal aspect, TF within the lesion (originating from the vessel wall) would have to diffuse through the deposited thrombus to the luminal surface to be present at the site of thrombus growth. Nemerson's group has indicated that it would take several hours for TF to diffuse from the vessel wall to the luminal site of thrombus expansion. TF derived from microparticles or leukocytes and deposited on the surface of the clot would not have to diffuse through the thrombus and therefore could rapidly enhance thrombus expansion. Reproduced with permission from Rauch and Nemerson (77).

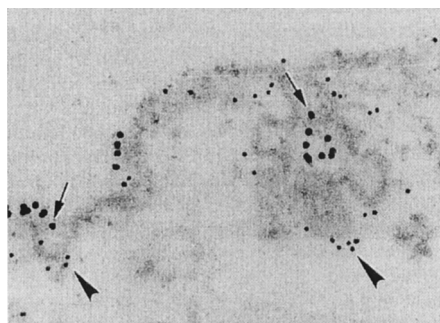


Figure 5. Electron micrograph of human smooth muscle cells immunogold-labeled with anti-tissue factor antibodies (arrows). Caveolae, 50–100 nm flask-shaped structures located at or near the plasma membrane, form when the protein caveolin-1 aggregates in a microdomain of a lipid raft. To define caveolae, the cells were washed and labeled with rabbit antibodies against caveolin and then, followed by gold-labeled anti-rabbit-IgG (arrowheads). Reproduced with permission from Mulder et al (83).

teraction between CD15 on leukocytes and P selectin (CD62) on platelets and to be inhibited by monoclonal antibodies to CD-15 and human TF (64). This raises the possibility of a novel approach to antithrombotic therapy on the basis of inhibition of ligands involved in leukocyte-platelet binding. Indeed, in an animal model, recurrent coronary thrombi were prevented by antagonism of the binding of P selectin to CD-15, demonstrating the importance of the interaction of these

membrane-bound carbohydrates in the transfer of inactive TF to its active state on activated platelets (88).

TF AS A RECEPTOR

A recent discovery has been the involvement of TF in cell signaling (Fig. 6). Binding of factor VIIa to cells expressing TF on their surface has been shown to elicit a transient increase in cytosolic calcium. This has been demonstrated in human umbilical vein endothelial cells stimulated with IL-1 β to express TF and in a human bladder carcinoma cell line (J82) that constitutively expresses high levels of TF (89). The increase has been shown to be dependent on the binding of catalytically active VIIa and IXa (90) at concentrations close to those found *in vivo* in human plasma (91).

The mechanism of signal transduction is unclear, but several investigators have demonstrated the importance of mitogen-activated protein kinases in this process (92–94). Recently, it has been shown that the TF–factor VIIa complex might signal through the protease-activated receptor (PAR) family (95) in a manner similar to thrombin. PAR-1, PAR-3, and PAR-4, but not PAR-2, are activated by thrombin. PAR-2 is expressed on vascular endothelial cells; however, its activators have not been fully characterized. One group of investigators has found that PAR-2 in *Xenopus* oocytes can be activated by the TF–factor VIIa complex or by factor Xa (96). By studying mutant forms of TF lacking the cytoplasmic carboxy tail, Camerer et al. (96) were able to show that PAR-2 activation occurred independently of the cytoplasmic domain. However, data have not been consistent and others have been unable to demonstrate signaling via PAR-1–4 on TF–factor VIIa exposure (97).

The consequence of TF signaling on gene expression and cell phenotype has been explored by a number of investigators. TF signaling has been shown to up-regulate the expression of a number of genes, including transcription regulators, growth factors, cytokines, and proteins involved in cellular migration. The transcriptional responses are abrogated by anti-TF antibodies and unaffected by hirudin (91).

OTHER ACTIONS OF TF

Leukocytes leave the bloodstream to enter sites of inflammation by squeezing

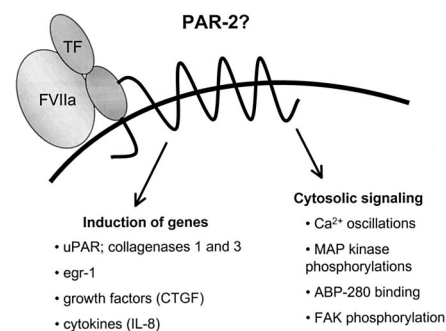


Figure 6. Activation of coagulation. ABP-280, actin binding protein 280; CTGF, connective tissue growth factor; egr-1, early growth response-1 gene; FAK, focal adhesion kinase; FVIIa, factor VIIa; IL-8, interleukin-8; MAP kinase, mitogen-activated protein kinase; PAR-2, protease-activated receptor-2; TF, tissue factor; uPAR, urokinase plasminogen activator receptor. Reproduced with permission from Siegbahn A: Cellular consequences on factor VIIa binding to tissue factor. *Haemostasis* 2000; 30(Suppl 2):41–47.

between tightly apposed endothelial cells. The factors controlling transmigration (diapedesis) are incompletely understood. Recently, TF has been shown to regulate the migration of peripheral blood monocytes through a confluent layer of human umbilical vein endothelial cells, suggesting a role for TF in monocyte diapedesis (98). TF has also been shown to regulate the angiogenic properties of tumor cells. *In vivo*, murine tumor cells transfected to overexpress TF grew more rapidly and established more vascular tumors than control transfectants (99).

TF PATHWAY INHIBITORS

TFPI-1 is the principal endogenous inhibitor of the TF pathway *in vivo*. TFPI-1, a serine protease inhibitor, inhibits factor Xa directly and the TF–factor VIIa catalytic complex in a factor Xa-dependent fashion. Activation of coagulation occurs when factor VII or VIIa present in plasma binds to TF, and the TF–factor VIIa complex then activates limited quantities of factor X and factor IX (100). With the generation of factor Xa, the inhibitory effect of TFPI becomes manifest and prevents further production of factors Xa and IXa by the TF–factor VIIa complex. Further generation of factor Xa is supported by the intrinsic pathway, involving factors VIIIa and IXa. A Kunitz-type inhibitor, TFPI-1 inhibits TF in a two-stage process. First, TFPI-1 binds and inactivates Xa (101). In the second phase,

TFPI-1 bound to Xa forms a quaternary complex with the TF-factor VIIa complex, thereby inhibiting thrombin generation and fibrin formation (Fig. 7) (102). TFPI-2, a homolog of TFPI-1, also inhibits the TF-factor VIIa complex, however, its role in the inhibition of the TF pathway *in vivo* is unclear (103). Although TFPI-1 and TFPI-2 share sequence homology and structure, they are, nonetheless, distinct proteins coded for by separate genes on different chromosomes (104, 105). TFPI-2 has also been found to occur in other animals, including *Escherichia coli* (106), mice (107), and baboons (108). Animal models of TFPI-1 depletion have been shown to be sensitized to TF and endotoxin and readily develop disseminated intravascular coagulation (109, 110). Further evidence of the importance of TFPI-1 comes from the observation that mice homozygous for TFPI gene-disruption of the Kunitz-1 domain (K1) uniformly perish *in utero* (111). Of these, 60% die between embryonic days E9.5 and E11.5, with signs of yolk sac hemorrhage. Of those surviving beyond E11.5, hemorrhage, particularly in the central nervous system and tail, was evident during later gestation, and none of the TFPI(K1)^{-/-} mice survived to the neonatal period. In these older embryos, the presence of immunoreactive fibrin in the liver and intravascular thrombi is consistent with the notion that unregulated factor VIIa/TF action and a consequent consumptive coagulopathy underlay the bleeding diathesis. It is probable that deficiency of TFPI in humans is similarly fatal *in utero*, and an individual with TFPI deficiency has not been identified.

Under normal conditions, the major pool of TFPI-1 is constitutively expressed by endothelial cells of the microvasculature but not by endothelial cells of larger vessels (112, 113). A small fraction of TFPI-1 is stored in platelets (114) or circulates in a free state in plasma (115). Fractionated and unfractionated heparin release full-length TFPI-1 from the endothelial surface after displacement from glycosaminoglycans in the glycocalyx (116). Recently, TFPI-1 has been found to co-localize with endothelial caveolae (117).

TFPI in Disease States

Activation of the TF/VIIa pathway is a key process in the pathologic up-regulation of the coagulation cascade during bacteremia and endotoxemia. In bacterial

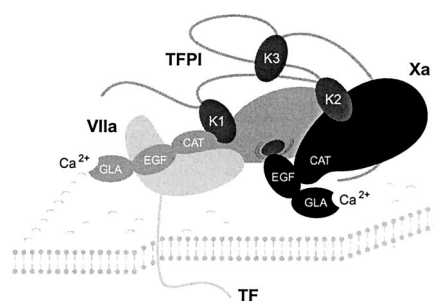


Figure 7. Hypothetical model of tissue factor pathway inhibitor's (TFPI's) binding to tissue factor (TF), factor VIIa, and factor Xa on the cell surface. Note that TF spans the cell membrane. CAT, catalytic domains for tissue factor and factor Xa; EGF, epidermal growth factor domains on tissue factor and factor Xa; GLA, gamma-carboxy glutamic acid domains on tissue factor and factor Xa; K1–3, Kunitz domains 1, 2, and 3 for TFPI. Reproduced with permission from Zhang et al (99).

sepsis, endotoxins induce the production of a number of proinflammatory cytokines: tumor necrosis factor- α , IL-1, IL-6, and IL-8. Cytokines and endotoxins stimulate the expression of TF on circulating monocytes and endothelial cells (51–55). In a recent study, TF was found to be significantly elevated in trauma patients with disseminated intravascular coagulation compared with healthy controls, whereas TFPI-1 was found to be within physiologic levels (118). Other reports have found low or elevated levels of TFPI-1 in disseminated intravascular coagulation (119, 120). Put together, these findings suggest that disseminated intravascular coagulation develops as a result of an imbalance between TF and its inhibitor, with high levels of TF insufficiently neutralized by TFPI-1.

Within human carotid atherosclerotic plaques, TFPI-1 is localized to endothelial cells, SMCs within the fibrous cap, and macrophages within the shoulder region. In one study, TFPI-1 within plaque was found to be biologically active, and inhibition of TFPI-1 with a polyclonal antibody resulted in an eight-fold increase in TF activity (114). Furthermore, examination of the relative activity of TF and TFPI-1 showed that TFPI-1 is associated with attenuated TF activity (121). In heterozygous TFPI-1-deficient mice, which are prone to atherosclerosis because of an apolipoprotein-E deficiency, the atherosclerotic area was increased and thrombosis after plaque injury occurred more quickly compared with controls (122). In a recent study, retroviral transfer of the

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gene coding for TFPI-1 was found to inhibit thrombus formation in stenotic and injured rabbit carotid arteries, whereas transfection of the contralateral carotid with a reporter gene had no effect on thrombosis (123). Collectively, these data demonstrate that an imbalance of TF/TFPI may promote or inhibit atherosclerosis and thrombosis.

STUDIES OF TFPI-1 IN ANIMAL MODELS AND HUMANS

Administration of human recombinant TFPI-1 (rTFPI-1) in animal models of severe sepsis has demonstrated a significant reduction in mortality (124–126), an improvement in markers of coagulopathy, and a reduction in inflammatory markers such as IL-6 (125, 127). Several small phase 1 and 2 studies of rTFPI-1 in humans with sepsis have indicated that rTFPI can be given safely, with no increase in bleeding and a trend toward a reduction in all-cause mortality (128, 129). In a recently completed multinational phase 2 study in 210 patients with severe sepsis, treatment with rTFPI reduced thrombin-antithrombin complexes and IL-6 levels, with no significant difference in adverse events (Fig. 7). The study was not powered for a mortality outcome but showed a trend toward a reduction in 28-day mortality (130). However, early reports indicate that the recently completed phase 3 trial of rTFPI-1 in severe sepsis, OPTIMIST, failed to show a reduction in the primary end point of 28-day all-cause mortality (Chiron, Emeryville, CA, press release, November 2001). The full data are awaited.

Studies of the effect of rTFPI on inflammatory markers have yielded inconsistent results. In models of induced endotoxemia in healthy subjects, rTFPI

completely inhibited activation of coagulation but had no effect on the proinflammatory markers IL-8, IL-6, tumor necrosis factor- α , macrophage inflammatory protein, or monocyte chemotactic protein (131, 132). The explanation for this discrepancy is unclear. However, models of induced endotoxemia in otherwise healthy human volunteers may not adequately reflect the inflammatory milieu of actual severe sepsis.

CONCLUSION

TF continues to be seen as the initiator of coagulation *in vivo*, although the intrinsic pathway is now understood to play a critical role in thrombus propagation and growth. Initiation of coagulation may occur through the exposure of tissue-bound TF or by the action of blood-borne TF on leukocytes and MPs. TF also seems to have a role outside coagulation, involving the regulation of monocyte diapedesis, cell signaling, and expression of growth factors. Although TF possesses a transmembrane domain and a cytoplasmic tail, most functions seem to be dependent on the extracellular catalytic domain. Cell signaling, calcium-dependent de-encryption, and rescue of transgenic TF null embryos do not require possession of an intracellular domain. Although early data from the phase 3 OPTIMIST study of rTFPI in severe human sepsis have not been encouraging, a rationale may exist for the use of rTFPI in vascular disease states such as unstable coronary syndromes, and further studies are warranted to investigate this potential.

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