In clinical practice, the activity of the extrinsic pathway is may play a role in both thrombus initiation and propagation. Several stimuli enhance TF gene expression in endothelial and vascular smooth muscle cells. In addition to these vascular cells, TF has recently been detected in the bloodstream of circulating cells such as leukocytes and platelets, as a component of microparticles, and as a soluble, alternatively spliced form of TF. Various cardiovascular risk factors like hypertension, diabetes, and dyslipidemia, increase levels of TF. In line with this observation, enhanced vascular TF expression occurs during atherogenesis, particularly in patients with acute coronary syndromes. (Circ J 2010; 74: 3–12)

Key Words: Cardiovascular diseases; Endothelium; Thrombosis

The Coagulation System

Under physiological conditions, the coagulation system is designed to maintain the circulating blood in a fluid state, but act towards restoration of vascular integrity by rapid clot formation after vessel injury. Historically, the coagulation cascade was divided into 2 major systems: the extrinsic and the intrinsic pathway (Figure 1).1 The tissue factor (TF)-factor VII/VIIa (FVII/FVIIa) complex was introduced as the extrinsic system as an exogenous factor (ie, TF) was required to activate circulating clotting factors. TF thereby initiates coagulation by interacting with activated factor FVII (FVIIa) to form the TF-FVIIa complex. In addition, TF is able to bind inactive factor VII (TF-FVII). FVIIa or the TF-FVIIa complex are able to convert the TF-FVII into its active form TF-FVIIa.

The intrinsic system contains the circulating components of the coagulation system including factor XII (FXII), XI (FXI), IX (FIX), and VIII (FVIII).2 Even though the 2 coagulation systems may be activated independently, they share a common pathway represented by the prothrombinase complex FVα-FXa (Figure 1).3 This complex catalyzes the conversion of prothrombin to thrombin, which cleaves fibrinogen into fibrin, activates platelets, and finally induces clot formation. Interestingly, stimulated platelets in a growing thrombus may additionally induce activation of coagulation factors; thus, there is evidence that the coagulation systems may play a role in both thrombus initiation and propagation.4 In clinical practice, the activity of the extrinsic pathway is measured by the prothrombin time, while the activated partial prothrombin time quantifies the function of the intrinsic system.

To maintain steady-state conditions, several inhibitory pathways may be activated to counteract the activity of the coagulation system. The serine protease inhibitor TF pathway inhibitor (TFPI) undergoes a quaternary complex with FXa, thus inhibiting the TF-FVIIa-complex.5 Activated protein C associates with protein S and inactivates FV and FVIII,6 while the anticoagulant protein antithrombin (ATIII) targets and inhibits thrombin as well as different clotting factors.7

Molecular Structure and Distribution of TF

The transmembrane glycoprotein TF (CD142, thromboplasmin) shares structural homology to class II cytokine receptors.8 The human TF gene is located on chromosome 1 (p21-p22). The 6 exons are translated into a 263-amino acid residue protein with a 219-amino acid extracellular domain, a 23-amino acid transmembrane region, and a 21-amino acid intracellular domain.9

Both vascular and non-vascular cells are known to express TF. In the vessel wall, TF is constitutively found in vascular smooth muscle cells (VSMCs), adventitial fibroblasts, and pericytes. The traditional notion that active TF is not present in the bloodstream was recently refused by the detection of functionally active TF in the circulation, so-called blood-borne or circulating TF (Figure 2).10,11
TF in the Vessel Wall

Endothelial Cells (ECs)
Expression of TF in ECs has been reviewed recently. Briefly, resting ECs express, if at all, very little TF in vitro and in vivo. Nevertheless, upon stimulation with cytokines (tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), or mediators like histamine, thrombin, oxidized LDL, or vascular endothelial growth factor, endothelial TF expression is induced (Figure 3). The above mentioned mediators stimulate, after binding to their corresponding receptor, the MAP kinases p38, ERK, and c-jun terminal NH₂-kinase with downstream activation of transcription factors such as NFκB, AP-1 or EGR-1. Also the protein kinase C as well as the Rho-Kinase pathway are known to mediate TF induction. In contrast, the PI3 kinase pathway and its downstream target Akt, GSK-3β, and the mammalian target of rapamycin in endothelial TF expression either at the transcriptional or the translational level. Additional posttranscriptional regulatory effects are known to play a role in endothelial TF expression; indeed, lipopolysaccharide (LPS) induce TF upregulation also via an increased TF mRNA stability.

VSMCs
In contrast to ECs, VSMCs constitutively express TF in vitro and in vivo with a hemostatic barrier. An induction of TF expression after stimulation with different mediators (TNF-α, CD40 ligand, histamine, thrombin, PDGF-BB, endotoxin, aggregated low-density lipoprotein (LDL), lysophosphatidic acid) has been described; recently, also C-reactive protein was found to induce TF expression both in vitro and in vivo.

Circulating (Blood-Borne) TF

Monocytes and Macrophages
Blood monocytes represent the predominant source of TF in the circulation. Similar to ECs, monocytes constitutively express little TF under basal conditions, but its expression can be further enhanced by specific stimuli including C-reactive protein, serum amyloid A, angiotensin II, oxidized LDL, TNF-α, and LPS. LPS, a key mediator in bacterial sepsis, is a potent inducer of monocyte TF both in vitro and in vivo. LPS induction leads to enhanced TF transcription via activation of all 3 MAP kinases; on the other hand, mRNA stability is impaired by LPS. Similar to ECs, activation of the PI3 kinase pathway exerts an inhibitory effect on TF induction in monocytes.

Granulocytes
Whether neutrophils express TF upon stimulation or not is still a matter of debate. Even though no TF-dependent procoagulant activity has been detected in whole blood stimulated with different agents, there is increasing evidence that neutrophils may be able to express TF under specific inflam-
Tissue Factor in the Cardiovascular System

For instance, the complement factor C5a induces TF expression in neutrophils both in vitro and in vivo\(^{47,48}\); thus pointing to a role of TF in the antiphospholipid-syndrome which is going on with an activated complement system. In addition to the de novo synthesis of TF in neutrophils, granulocytes may take up TF via TF-positive microparticles (MPs).\(^{49}\)

In contrast, in eosinophilic granulocytes and their progenitors cells, basal TF expression has been described.\(^{50}\) Stimulation with granulocyte-macrophage colony-stimulating factor or platelet-activating factor, results in de novo TF transcription and translocation from the granules to the cell membrane\(^{50}\); thus contributing to the pro-thrombotic state in hypereosinophilic syndromes.\(^{51}\)

Platelets

Similar to granulocytes, TF expression in platelets is still controversially discussed. While some authors neither detected TF antigen nor activity in resting as well as calcium ionophore- or collagen-stimulated platelets,\(^{52}\) others described functional TF in platelets.\(^{53-57}\) Indeed, different mechanisms of TF expression in platelets have been described, underscoring a potential role of platelet TF. Translocation and activation of existing TF protein from intracellular compartments to the platelet surface, uptake of TF from other sources, mainly via MPs, and de novo TF mRNA and protein synthesis have been proposed (Figure 4).

Muller et al\(^{53}\) found TF in different areas of resting platelets: on the membrane, in the matrix of \(\alpha\)-granules, and in the

**Figure 2.** Circulating (blood-borne) tissue factor (TF). TF in the circulation is present as a cellular and a non-cellular forms. Cellular circulating TF has been found in monocytes, neutrophil and eosinophil granulocytes, as well as in platelets. Non-cellular TF is either present as the so-called alternatively spliced TF or as a component of microparticles.

**Figure 3.** Induction of tissue factor (TF) protein expression in human endothelial cells. Under resting conditions, human endothelial cells only express, if at all, very little amounts of TF. Upon stimulation with tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) (5ng/ml), histamine (10\(^{-5}\)mol/L), or thrombin (1U/ml) for 5h enhances TF protein expression. Protein loading was controlled by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) staining.
open canalicular system. Stimulation with different agonists finally resulted in the presentation of TF on the platelet surface.

Due to the fact that human megakaryocytes neither express TF mRNA nor protein, there was the hypothesis that growing platelets may take up TF only from other cells. Indeed, TF-containing MPs, shed from monocytes and possibly polymorphonuclear leukocytes, are being taken up by platelets via a CD15 and P-selectin dependent interaction.\(^{56,58}\) In addition, activated ECs release TF-containing MPs, which could potentially be transferred to platelets as well.\(^{59,60}\)

Recent studies confirmed the possibility for true de novo TF protein expression in platelets upon stimulation by using metabolic radiolabeling.\(^{54}\) ADP, a potent inducer of platelet activation, enhanced platelet TF activity.\(^{61}\) Schwertz and colleagues did not detect TF mRNA in resting platelets, but revealed the presence of TF pre-mRNA which is spliced into TF mRNA upon stimulation with different agonists,\(^{62}\) resulting in increased TF protein expression and activity.

**MPs**

TF antigen and activity has been detected in human platelet-free plasma. The majority of this TF is located in MPs. TF containing MPs are submicrometric fragments with a diameter of 0.1–1 μm released from activated or apoptotic eukaryotic cells; depending on their origin, they differ in antigen and phospholipids composition. MPs need to be distinguished from exosomes, which originate from intracellular compartments, are smaller (<0.1 μm), and differ in their composition. The main source of circulating MPs\(^{63,64}\) are monocytes and platelets, but other vascular cells like ECs,\(^{65}\) VSMCs,\(^{66}\) and eosinophils\(^{50}\) also release TF containing MPs. Levels of TF-containing MP are elevated in patients with cardiovascular risk factors such as diabetes, hypertension, obesity, and dyslipidemia, but their specific role is still debated. In vitro thrombin generation induced by TF containing MPs was confirmed;\(^{67}\) nevertheless, its role in thrombus formation in vivo remains to be delineated. MPs may play a role in thrombus propagation; indeed, a growing thrombus separates TF produced at the vessel wall from circulating coagulation factors. Under these conditions, TF-containing MPs in the blood stream may still bind to activated platelets through interaction of PSGL-1 (on the MPs) and P-selectin (on the platelet surface).\(^{10}\)

**Alternatively Spliced TF**

The full-length TF mRNA contains 6 exons. Splicing exon 4 directly to exon 6 creates the soluble alternatively-spliced form of TF, a protein lacking the transmembrane domain of TF and therefore missing the membrane anchorage\(^{68}\) (Figure 5). Its expression and release from ECs as well as cardiac myocytes is induced by cytokines;\(^{69}\) further, asTF has been detected in organs such as lung or placenta, and in different cancers. Recently, the PI3 kinase has been shown to reduce TNF-α-induced asTF mRNA expression in ECs.\(^{70}\) The contribution of asTF to total plasma TF activity as well as its impact on arterial thrombus formation still needs to be determined. Similar to TF containing MPs, asTF may contribute to thrombus growth as much as MP-derived TF. Nevertheless, it is still uncertain whether asTF exerts procoagulant activity or not. Censarek et al published that asTF do not exhibit clotting activity,\(^{71}\) while others identified that

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**Figure 4.** Platelet tissue factor (TF). Potential expression pathways and origin of TF in platelets. Platelets either express TF de novo via splicing of a TF pre-mRNA or may take up TF from microparticles originating from other cellular sources like leukocytes. Finally, TF protein stored in α-granules may be transferred to the cell surface and thereby become activated.
Figure 6. Thrombus formation after endothelial injury. Endothelial injury on top of an atherosclerotic plaque leads to the exposure of highly pro-coagulant material containing tissue factor (TF). During thrombus initiation, rapid binding of platelets to the subendothelial matrix and activation of the coagulation cascade occurs. Further processes (during thrombus propagation) include binding of additional platelets and activation of the coagulation cascade resulting in fibrin production. Lastly, the thrombus is stabilized by fibrin, which binds to glycoprotein Iib/IIa-receptors on platelets.
procoagulant activity of asTF depends on the presence of phospholipids. Apart from its role in thrombosis, asTF enhances migration and differentiation, but not proliferation, of ECs.

**TF Expression is Not TF Activity**

It is known that only a small amount of TF exerts pro-thrombotic activity. The presence of an inactive form (encrypted or cryptic) of TF may account, at least in part, for this observation. Recent studies imply that the disulfide bond between Cys and Cys is important for the coagulation activity of TF. The protein disulfide isomerase (PDI) disables coagulation by splitting this bond, while oxidation of these 2 cysteins by HgCl increases TF activity by formation of the disulfide bond. Surprisingly, PDI enhanced the coagulation activity of soluble TF, and in an in vivo carotid artery ligation model, PDI promoted thrombus formation. Further investigations are therefore required to elucidate the mechanisms involved in the regulation of TF activity in vivo.

**Role in Hemostasis**

Hemostasis represents a physiological response to injury to maintain vascular integrity and limit blood loss. In normal blood vessels, TF is present mainly in medial and adventitial cells including VSMCs and fibroblasts as well as pericytes, while ECs do not express TF under physiological conditions. Hence, the endothelium, which covers the lumen of all blood vessels, prevents the interaction of TF with circulating clotting factors and thereby exerts important antithrombotic properties. However, endothelial damage leads to the exposure of subendothelial TF to the bloodstream and in turn to the initiation of coagulation. Therefore, vascular TF was referred to as a “hemostatic envelope.” The essential role of TF in hemostasis is reflected by the observation that mice lacking TF die due bleeding complications at the embryonic stage. Similarly, humans can not survive without TF. Furthermore, the higher TF antigen level in vital organs such as the brain, heart, and lung as compared to skeletal muscle and joints underscores the pivotal role of TF in hemostasis.

**TF and Thrombosis**

Thrombosis is an inappropriate response to vascular wall injury in order to limit blood loss. Indeed, protection by an intact endothelium inhibited arterial thrombus formation. This injury results in an exposure of clotting factor to the subendothelial matrix and subsequently to thrombus formation. Formation of a clot at the site of vascular injury can be divided into 3 phases, ie, thrombus initiation, propagation, and stabilization (Figure 6). A pivotal role of TF in general within this process is widely accepted; indeed, inhibition of TF by anti-TF antibodies or application of TFPI reduce thrombus formation in different thrombosis model. However, depending on vessel anatomy (arteries vs veins), TF from different sources may be differently involved. While vessel injury and therefore exposure of subendothelial TF from the vessel wall is essential in arterial thrombosis, circulating TF, mainly associated with MPs, seems to be more important in venous thrombosis.

Day and colleagues described the role of vessel wall TF in a mouse carotid artery model. By genetically reducing either TF in all cells of the vessel wall or knocking out specifically TF in VSMCs, time to thrombotic occlusion was prolonged. Interestingly, selective blockade of TF in circulating cells did not affect thrombosis in the same model, suggesting a pivotal role of vessel wall TF in thrombus formation at least in large conduit arteries. In line with this, disruption of atherosclerotic plaques leads to exposure of TF to the blood resulting in formation of an occlusive thrombus (Figure 6), and active-site inhibited FVIIa inhibits thrombus formation on top of a ruptured plaque supporting the role of vessel-wall TF in arterial thrombosis.

In venous thrombosis, stasis of the blood stream is more important to induce thrombosis than vessel damage; thus, circulating TF should play a crucial role under these conditions. Indeed, in a jugular vein model using collagen-coated cotton thread, inhibition of TF reduced thrombosis even without endothelial injury. Surprisingly, hematopoietic cell-derived TF did not contribute to thrombus formation in an inferior vena cava mouse model of venous thrombosis. Further studies are therefore necessary to elucidate the role of TF in venous thrombosis.

**Effect of Cardiovascular Risk Factors on TF Expression**

Hypertension, dyslipidemia, and diabetes mellitus (DM) represent major atherothrombotic risk factors. In addition, they are leading to elevated levels of circulating TF antigen and activity as well as increased TF expression in atherosclerotic plaques, while pharmacological treatment of these conditions reduces TF.

**Hypertension**

Both short term as well as longstanding high blood pressure enhances TF. Hypertension increases shear stress leading to activation of NfκB and Egr-1, and subsequently to upregulation of TF gene expression. Angiotensin-converting-enzyme (ACE) inhibitors downregulate TF expression in vivo, while angiotensin II upregulates it in vascular cells via the angiotensin II type 1 receptor.

**Dyslipidemia**

Similarly, in patients presenting with elevated levels of LDL, TF activity is increased. In vitro oxidized LDL (oxLDL) enhances TF expression in vascular cells, while high-density lipoproteins (HDL) reduces it via activation of the PI3-kinase and/or the production of NO. Statins reduce TF expression and activity both in vitro and in vivo; interestingly, the drugs also inhibit TF expression in the absence of a cholesterol-lowering effect, suggesting a pleiotropic effect independent of the lowering cholesterol properties.

**DM**

In diabetes, TF expression is increased either directly by elevated glucose levels, hyperinsulinemia, or alternatively via activation of NfκB through advanced glycation end-products (AGE) or reactive oxygen species (ROS). AGE stimulates NfκB via their membrane receptor (RAGE) while ROS directly activate the transcription factor. Glycemic control reduces TF levels, and drugs used in the treatment of type 2 diabetes mellitus like rosiglitazone, a peroxisome proliferator-activated receptor γ agonist, downregulate TF expression.
**Atherosclerosis and Acute Coronary Syndromes (ACS)**

**TF and Atherosclerosis**
Inflammation and endothelial dysfunction play key roles in the development of atherosclerosis,[10,105] In healthy arteries, TF is expressed in fibroblasts, pericytes and in smooth muscle cells.[29,79] TF mRNA and antigen are detected in plaque macrophages early in atherogenesis,[29] while in later stages, also endothelial and smooth muscle cells as well as foam cells express TF.[29,106] Furthermore, TF is present extra-cellularly in MPs surrounding cholesterol clefts and within the necrotic core.[29,106,107] Nevertheless, its role in the pathogenesis of atherosclerosis is still uncertain. Even though TF has been shown to promote important processes of atherogenesis such as migration and proliferation of VSMCs[10,109] and angiogenesis,[29] its causal role is still controversial. Tilley et al used heterozygous TF knockout mice, which exhibit either 50% reduction in vascular TF activity or a 90% reduction in hematopoietic cell-derived TF, and investigated the development of atherosclerotic lesions. Interestingly, equal amount of atherosclerotic lesions were observed in these mice,[110] raising the question whether TF, at least in rodents, plays a role in atherogenesis, or not. This question still needs to be further investigated.

**TF in ACS**
Coronary atherectomy specimens of patients presenting with unstable angina or myocardial infarction, exhibit higher TF antigen and activity levels than those from patients with stable angina.[111,112] Also, higher plasma TF levels are found in patients with ACS as compared to controls.[113] Most probably, plaque rupture contributes to the elevated blood levels of TF in ACS, as this event leads to the exposure of highly procoagulant plaque content to the circulation.[107] This observation is supported by the fact that the level of TF seems to be associated with the severity of ACS, since patients presenting with unstable angina or non-ST-elevation myocardial infarction and a high TIMI score (≥4) exhibit higher TF than those with low TIMI score (<3).[114]

**Conclusions**
Thrombotic events are crucial for outcome in ACS, thrombembolism, and sepsis. A major effort has been undertaken to understand the biological mechanisms behind coagulation. TF, a transmembrane glycoprotein, is the key trigger of the coagulation cascade. Cytokines, growth factors, and biogenic amines induce TF expression in ECs and VSMCs; more recently, circulating TF has been detected in platelets, leukocytes, and MPs. As the key initiator of the coagulation cascade, TF plays an important role in hemostasis and thrombus formation; nevertheless, the relative contribution of vessel wall-associated vs circulating TF to both thrombus formation and propagation is still uncertain. Cardiovascular risk factor such as hypertension, dyslipidemia, and diabetes, enhance TF, while drugs like ACE inhibitors or statins decrease TF. These diseases are pro-atherogenic, and elevated levels of TF are found in atherosclerotic plaques. Nevertheless, whether TF is causally associated with atherogenesis is still a matter of debate.

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None.

**References**


Chemotherapy-induced thrombin generation via procoagulant endothelial microparticles is independent of tissue factor activity.


73. Bach RR. Tissue factor encryption.


86. Felmeden DC, Spencer CG, Chung NA, Belgoro FM, Blann AD, Bevers DG, et al. Relation of thrombogenesis to systemic hypertension to angiogenesis and endothelial damage/dysfunction (a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]). Am J Cardiol 2003; 92: 400 – 405.


