Tissue factor: beyond coagulation in the cardiovascular system

Alexander BREITENSTEIN∗†‡, Giovanni G. CAMICI∗† and Felix C. TANNER∗†‡

∗Cardiovascular Research, Physiology Institute, University of Zurich, Zurich, Switzerland, †Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland, and ‡Cardiology, Cardiovascular Center, University Hospital Zurich, Zurich, Switzerland

ABSTRACT

TF (tissue factor) is the main trigger of the coagulation cascade; by binding Factor VIIa it activates Factor IX and Factor X, thereby resulting in fibrin formation. Various stimuli, such as cytokines, growth factors and biogenic amines, induce TF expression and activity in vascular cells. Downstream targets of these mediators include diverse signalling molecules such as MAPKs (mitogen-activated protein kinases), PI3K (phosphoinositide 3-kinase) and PKC (protein kinase C). In addition, TF can be detected in the bloodstream, known as circulating or blood-borne TF. Many cardiovascular risk factors, such as hypertension, diabetes, dyslipidaemia and smoking, are associated with increased expression of TF. Furthermore, in patients presenting with acute coronary syndromes, elevated levels of circulating TF are found. Apart from its role in thrombosis, TF has pro-atherogenic properties, as it is involved in neointima formation by inducing vascular smooth muscle cell migration. As inhibition of TF action appears to be an attractive target for the treatment of cardiovascular disease, therapeutic strategies are under investigation to specifically interfere with the action of TF or, alternatively, promote the effects of TFPI (TF pathway inhibitor).

INTRODUCTION

Over the last few decades, cardiovascular disease has turned into a major public health burden accounting for the majority of deaths in the Western civilization. TF (tissue factor) is an important protein not only as the key trigger of the coagulation cascade, but also as a mediator in the pathogenesis of cardiovascular disorders. Hence therapeutic strategies specifically targeting TF and its effectors are under investigation. In the present review, we will focus on the regulation of TF expression, its role in cardiovascular diseases and the resulting implications for its therapeutic targeting.

TF EXPRESSION

TF (CD142) or thromboplastin, is a 47 kDa transmembrane glycoprotein with structural homology to class II cytokine receptors [1]. TF is widely expressed in both vascular smooth muscle cell.

Key words: cardiovascular disease, coagulation cascade, endothelial cell, fibrin, risk factor, tissue factor, vascular smooth muscle cell.

Abbreviations: AGE, advanced glycation end-product; AngII, angiotensin II; ApoE, apolipoprotein E; CRP, C-reactive protein; EGR-1, early growth-response gene product-1; ERK, extracellular-signal-regulated kinase; FIX, Factor IX; FXI, Factor VII; FVIIa, activated FVII; FX, Factor X; FXa, activated FX; GSK-3β, glycogen synthase kinase-3β; IL, interleukin; JNK, c-Jun N-terminal kinase; LDL, low-density lipoprotein; LPA, lysophosphatidic acid; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MP, microparticle; mTOR, mammalian target of rapamycin; NAPc2, nematode anticoagulant protein c2; NF-κB, nuclear factor-κB; oxLDL, oxidized LDL; PAR, protease-activated receptor; PDGF-BB, platelet-derived growth factor-BB; PDI, protein disulfide isomerase; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; RAGE, receptor for AGEs; rNAPc2, recombinant NAPc2; TF, tissue factor; asTF, alternatively spliced TF; TFPI, TF pathway inhibitor; rTFPI, recombinant TFPI; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor.

Correspondence: Professor Felix C. Tanner (email felix.tanner@access.uzh.ch).
The induction of TF expression is exemplified in an endothelial cell. Different mediators induce TF expression via stimulation of their corresponding cell-surface receptors.

TF is present intracellularly, on the cell surface or may be shed (so-called soluble TF). H1R, histamine H1 receptor; 5-HT2A, 5-hydroxytryptamine-2a receptor; IL1-R, IL-1 receptor; KDR, VEGF receptor 2; TNF-R, TNF-α receptor; TLR, Toll-like receptor.

vascular and non-vascular cells, in the latter usually at higher levels. In cells with continuous contact with the bloodstream, such as endothelial cells, monocytes and platelets, TF is either expressed at a very low level or in an inactive (so-called encrypted) form; nevertheless, stimulation of these cells by various mediators induces TF protein expression and activity in vitro. Constitutive expression of TF in normal blood vessels is mainly observed in medial and adventitial cells, especially in vascular smooth muscle cells, adventitial fibroblasts and pericytes. Hence the endothelium, which lines the lumen of blood vessels, prevents the contact of TF-expressing cells with the flowing blood and thereby intravascular clotting; however, damage to the vessel wall or activation of endothelial cells can rapidly trigger coagulation. Under these circumstances, TF acts as a primary mediator limiting blood loss and restoring vascular integrity by providing a first haemostatic barrier after vessel injury.

**Cellular TF**

**Endothelial cells**

Under physiological conditions, endothelial cells express only very little TF if at all. Nevertheless, cytokines, such as TNF-α (tumour necrosis factor-α) [2] or IL-1β (interleukin-1β) [3], and mediators including CD40 ligand [4], serotonin [5], histamine [6], thrombin [7], oxLDL (oxidized LDL (low-density lipoprotein)) [8] or VEGF (vascular endothelial growth factor) [9] are able to induce TF expression in vitro (Figure 1) by binding to their corresponding receptors. This stimulation leads to the activation of the MAPKs (mitogen-activated protein kinases) p38, ERK (extracellular-signal-regulated kinase) and JNK (c-Jun N-terminal kinase) with subsequent activation of transcription factors such as NF-κB (nuclear factor κB), AP-1 (activator protein-1) or EGR-1 (early growth-response gene product-1) [4,10,11] (Figure 2). In the case of NF-κB, MAPK activation leads to degradation of the inhibitory protein IκB (inhibitor of NF-κB), thereby enabling nuclear translocation of NF-κB. TNFα, histamine and thrombin induce TF expression via activation of all three MAPKs [2,6,7,11,12], whereas the effect of VEGF is regulated by p38 and ERK only [11]. In addition to the MAPKs, PKC (protein kinase C) is also involved in TF induction by TNF-α and VEGF [11], whereas thrombin also activates TF expression through the Rho-kinase pathway [7,13].

In contrast with MAPKs and PKC, a negative regulation of TF expression has been recognized for the PI3K (phosphoinositide 3-kinase) pathway (Figure 2). Stimulation of endothelial cells with TNF-α, histamine, thrombin or VEGF inhibits PI3K in parallel to MAPK activation, leading to an enhanced TF expression [7,14–16]. To date, the mechanism involved in the PI3K-mediated inhibition of TF expression has not been elucidated in detail. It is known that downstream targets of PI3K, such as Akt and GSK-3β (glycogen synthase kinase-3β), are involved; indeed, Akt inhibits MAPK activation [14] and GSK-3β regulates TF expression at the transcriptional level [17]. In contrast, mTOR (mammalian target of rapamycin) and p70S6 kinase inhibit TF translation [15,18]. In addition to these signal transduction events,
Figure 2  Signalling mechanisms involved in TF regulation

Induction of TF is regulated at different levels of protein expression. Various mediators induce TF expression through activation of their receptors. Stimulation of MAPKs (p38, JNK and ERK) and PKC activates transcription factors, resulting in an induction of TF mRNA and protein expression. In contrast, activation of the PI3K pathway inhibits TF induction either at the transcriptional or post-transcriptional level. PIP$_2$, phosphatidylinositol 4,5-bisphosphate; PIP$_3$, phosphatidylinositol 3,4,5-triphosphate; PKC$_{I/A}$, inactive/active PKC; p70S6, p70S6 kinase.

post-transcriptional regulatory effects may contribute to increased TF expression as well. For example, LPS (lipopolysaccharide)-induced TF up-regulation is mainly a result of increased TF mRNA stability, rather than an increased rate of transcription [19].

TF protein expression and pro-coagulant activity do not necessarily need to correlate [9]. It is generally accepted that only a relatively small amount of cellular TF is present on the cell surface and, therefore, active and able to initiate thrombus formation [20,21]. Concomitant expression of TFPI (TF pathway inhibitor), the endogenous inhibitor of TF, may interfere with TF activity and at least in part accounts for this observation. The presence of an inactive form of TF at the cell surface, so-called encrypted or cryptic TF, may contribute as well, although the processes involved in encryption and decryption of TF are not yet completely understood. An early study implied that the disulfide bond between Cys$^{186}$ and Cys$^{209}$ is critical for coagulation, and that PDI (protein disulfide isomerase) disables coagulation by directly targeting this bond [22]. Consistent with this interpretation, oxidation of these two cysteine residues by HgCl$_2$, an oxidizing agent, increases TF activity by the formation of the disulfide bond [23]. Nevertheless, previous studies have raised doubts about the role of PDI in regulating the coagulation activity of TF in vitro [24]; the coagulation activity of soluble TF may even be enhanced by PDI [25]. Most recently, it has been observed that PDI promotes TF-dependent thrombus formation in a carotid artery ligation model in vivo [26]. In summary, the idea that alterations in the redox state regulate TF activity is very interesting; nevertheless, further investigation is necessary to elucidate the mechanisms involved in detail and to clarify the potential in vivo relevance.

Vascular smooth muscle cells

Vascular smooth muscle cells constitutively expressing TF reside in the medial layer of normal arteries and provide the vessel with a haemostatic barrier after injury [27]. In culture, low levels of TF expression can be observed in vascular smooth muscle cells under basal conditions [28–32], whereas an up-regulation of TF expression after stimulation with mediators such as TNF-α, CD40 ligand, histamine, thrombin, PDGF-BB (platelet-derived growth factor-BB), endotoxin, aggregated LDL, LPA (lysophosphatidic acid) and CRP (C-reactive protein) [6,28,30,33–38] is described. In contrast with endothelial cells, the mechanisms mediating TF protein expression are poorly investigated in vascular smooth muscle cells. Nevertheless, thrombin has been shown to induce TF expression in this cell type via activation of p38 and PI3K [35], whereas LPA [37], PDGF-BB [34] and CRP [38] stimulate TF induction through ERK activation in vascular smooth muscle cells.

TF antigen is found in three different pools in vascular smooth muscle cells, namely at the cell surface, as
Circulating (blood-borne) TF

The presence of active TF in circulating blood contrasts with the traditional belief that TF simply represents a haemostatic barrier after vessel injury [40,41]. Blood-borne TF is found in monocytes, eosinophils, platelets, MPs (microparticles) and as a soluble splicing variant of TF. Even though the importance of blood-borne TF is emerging, its role in arterial thrombosis and haemostasis is still debated.

Monocytes and macrophages

Monocytes represent a major source of TF in circulating blood. Even though these cells constitutively express some TF under basal conditions, its expression is up-regulated after stimulation with CRP [42,43], SAA (serum amyloid A) [44], CD40 ligand [45], PDGF-BB [46], AngII (angiotensin II) [47,48], oxLDL [49,50] and TNF-α [32,51]. The most widely studied stimulus in this cell type is LPS [10,52,53]; indeed, DIC (disseminated intravascular coagulation), a severe complication of sepsis, is associated with multiple intravascular thrombotic events secondary to enhanced TF expression [54–56]. LPS-induced TF expression is mediated through p38, ERK and JNK [52,53], leads to a translocation of the transcription factors EGR-1, c-Fos/c-Jun and NFκB into the nucleus, and thereby to an activation of the TF promoter [10]. In addition to enhancing TF transcription, LPS also impairs TF mRNA stability [57]. In line with observations in endothelial cells, activation of the PI3K pathway exerts an inhibitory effect on TF induction in monocytes [52].

Activation of monocytes may also occur through many other pro-inflammatory mediators secreted mainly from Th1 as opposed to Th2 T-cells such as TNF-α [32,51] and IFN-γ (interferon-γ) [58,59]. Th1-cell-derived cytokines indeed induce monocyte TF expression; moreover, direct cell-to-cell contact with Th1 B-cells induces TF expression as well [60]. In contrast, Th2 cells prevent Th1-induced TF expression via the secretion of IL-4, IL-10 and IL-13 [60]. Transformation of monocyte-derived macrophages into foam cells also results in increased TF expression [61].

Granulocytes

Mature blood eosinophils as well as their progenitor cells have been found to express TF under resting conditions [62]. In contrast, resting neutrophils and basophils express only very low levels of TF [63]. After stimulation with GM-CSF (granulocyte/macrophage colony-stimulating factor) or PAF (platelet-activating factor), TF in resting eosinophils is translocated from the granules to the cell membrane, and, in addition, TF transcription is increased [62]. These findings may be relevant not only for the pro-thrombotic status of hyper-eosinophilic diseases, but also for the onset of vascular permeability leading to oedema [64].

Even though no TF procoagulant activity can be detected in inactivated neutrophils [63] or in stimulated whole blood [65], there is increasing evidence that neutrophils may be able to express TF under specific inflammatory conditions [66–68].

Platelets

The role of platelet-derived TF is still a subject of controversy, since some authors have failed to identify TF in platelets [69]. Nevertheless, TF-containing platelets have been identified by several investigators [63,70–73]. In resting platelets, TF is found at the membrane and in the matrix of α-granules as well as in the open canicular system [63], whereas stimulation with different agonists leads to the presentation of TF on the platelet surface [63]. Despite this evidence, the origin of platelet TF is still controversial. Because neither TF mRNA nor protein are detectable in human megakaryocytes, it was initially hypothesized that platelets acquire TF from other cells. Indeed, circulating monocytes and possibly PMN (polymorphonuclear) leukocytes [74] are a source of platelet TF, since TF-containing microparticles derived from these cells fuse and transfer TF to platelets through a CD15- and P-selectin-dependent interaction [72,74]. Other studies have shown that activated endothelial cells release TF-containing microparticles, which could potentially be transferred to platelets as well [75,76]. Previous observations instead revealed the presence of TF mRNA in activated human platelets [77], most probably a splicing variant of a TF pre-mRNA, detected immediately after stimulation with different platelet agonists [78]. Furthermore, by using metabolic radiolabelling, de novo TF protein expression in platelets was confirmed [70], and stimulation with ADP induced a significant increase in platelet TF activity [77].

Microparticles

TF-containing MPs are vesicles with a diameter of 0.1–1 μm released from either activated or apoptotic eukaryotic cells. Platelets and monocytes represent a major source of circulating MPs [79,80], but endothelial cells [81,82], vascular smooth muscle cells [83] and eosinophils [62] have been shown to release TF-containing MPs as well. Nevertheless, the role of such TF-containing MPs in arterial thrombus formation is still debated. Even though thrombin formation by MP-associated TF was confirmed in vitro [84], its role in thrombus initiation in vivo is still unclear. In one model, vessel-wall-derived TF was described to act as the primary mediator of thrombus formation after vascular injury [41], whereas blood-borne TF determined thrombus formation in another model [40].

encrypted TF, and intracellularly [28,39]. In line with this, TF activity only reflects TF expression to some extent.
**TF FUNCTION**

FVIIa [activated FVII (Factor VII)] interacts with TF to build the bimolecular TF–FVIIa complex (Figure 3), initiating the coagulation cascade. Apart from this, TF is able to bind inactive FVII, leading to the TF–FVII complex, which in turn can be converted into TF–FVIIa by either FVIIa or the TF–FVIIa complex. Via activation of FIX (Factor IX), FX (Factor X) will subsequently be activated, resulting in the conversion of prothrombin into thrombin with the help of FVa (activated Factor Va) and Ca^{2+}. Finally, the coagulation cascade leads to fibrin deposition, platelet activation and thrombus formation. In an auto feedback loop, several of these activated proteases, including FIXa (activated FIX), FXa (activated FX), thrombin and the TF–FVIIa complex itself, can convert FVII into FVIIa [89].

Apart from its well-known role in haemostasis, the TF–FVIIa complex regulates a broad range of cellular responses; these intracellular mechanisms are comprehensively reviewed by other authors [90–92]. Briefly, such responses are either mediated by TF itself depending on its cytoplasmatic domain, or, alternatively, by FVIIa through PARs (protease-activated receptors). TF indeed increases VEGF expression in culture [93] and plays a role in angiogenesis [94], the development of embryonic blood vessels [95], and the progression of cancer growth and metastasis [96–98]. Furthermore, TF participates in the regulation of vascular smooth muscle cell migration [99]. Vascular remodelling is impaired in mice lacking the cytoplasmatic domain of TF, implying an involvement of this domain in regulating vascular smooth muscle cell migration [100]. Other non-coagulant responses to the TF–FVIIa complex are

**asTF (alternatively spliced TF)**

The full-length form of TF mRNA contains 6 exons and codes for a 263-amino-acid residue TF protein containing an extracellular, a transmembrane and a cytoplasmic domain. asTF is generated through splicing of exons 4–6 resulting in a frame-shift mutation; this isoform lacks membrane anchorage and is therefore soluble [85]. Cytokines stimulate its expression in and release from endothelial cells [86] and cardiac myocytes; furthermore, asTF has been detected in plasma, lung, placenta and different cancer cells. As a distinguishable source of circulating TF, it may have an important role in thrombus propagation [85,86], as vessel-wall-associated TF, being separated from the bloodstream by the freshly formed thrombus, may be prevented from contributing to thrombus growth [40]. Nevertheless, it is still debated whether asTF has pro-coagulant activity [87]. In addition to thrombus formation, asTF promotes migration and differentiation, but not proliferation, of endothelial cells, indicating a potential role in angiogenesis [88].

**Figure 3  TF is the key initiator of the coagulation system**

FVIIa and TF build a complex leading to the activation of FIX and FX. Finally, this results in thrombin generation and clot formation. The physiological inhibitor of TF, TFPI, binds to FXa and thereby inhibits TF–FVIIa activity.
mediated mainly through PAR2 and to a lesser degree PAR1 [96,101–103]; these actions appear to be regulated by FVIIa, independent of the cytoplasmatic domain of TF, and include the expression of pro-inflammatory and pro-angiogenic mediators in different cell lines [104–107].

**TF IN METABOLIC AND CARDIOVASCULAR DISEASES**

**TF and diabetes mellitus**

Plasma levels of TF activity [108], TF antigen [109] and TF-containing MPs [110] as well as TF expression in monocytes [108] are elevated in diabetic patients compared with normoglycaemic control subjects. Consistent with this observation, experimentally induced hyperglycaemia increases TF antigen and activity in both plasma and monocytes from healthy humans [111,112]. Furthermore, high glucose levels increases thrombin- and IL-1-induced TF expression in human endothelial cells [113]. In diabetes, hyperglycaemia induces the formation of AGEs (advanced glycation end-products) up-regulating TF antigen through their membrane receptor RAGE (receptor for AGEs) [114]; conversely, blockage of this receptor suppresses TF plasma levels. Moreover, AGEs induce TF in both endothelial cells and monocytes via RAGE and activation of its downstream target NF-κB [115–118]. Improving glycaemic control in diabetic patients reduces plasma TF levels, and drugs applied for treating diabetes such as rosiglitazone, a PPAR-γ (peroxisome-proliferator-activated receptor-γ) agonist, down-regulate TF protein expression [119].

**TF and dyslipidaemia**

The incidence of arterial thrombosis is enhanced in patients with dyslipidaemia and dyslipoproteinaemia [120]. Human subjects with elevated LDL levels have an increase in TF plasma activity [121], which may be related to the observation that oxLDL enhances TF expression in monocytes/macrophages [49], endothelial cells [8,122] and vascular smooth muscle cells [39]. On the other hand, reconstituted HDL (high-density lipoprotein) blunts thrombin-induced TF expression in endothelial cells by stimulating the PI3K pathway [16] and/or by increasing the production of NO [123]. Statins are HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA reductase) inhibitors and are widely used in the treatment of dyslipidaemia; these agents reduce TF expression in monocytes [124], endothelial cells [7] and vascular smooth muscle cells [125]. In a human endothelial cell line, statins inhibited TF activity by altering membrane phosphatidylserine, leading to TF encryption rather than an impaired TF protein expression [126]. These in vitro findings are consistent with the observation that simvastatin and rosuvastatin impair TF expression in advanced atherosclerotic lesions of ApoE (apolipoprotein E)-deficient mice in the absence of a cholesterol-lowering effect [127,128].

**TF and hypertension**

In patients with arterial hypertension, elevated plasma levels of TF antigen are observed compared with normotensive control subjects [129]; furthermore, antihypertensive treatment reduces TF plasma levels in these patients [129,130]. Hypertension enhances chronic endothelial shear stress, leading to activation of NF-κB and, in turn, the up-regulation of TF expression [131]. Moreover, AngII increases TF expression in different human vascular cells, including monocytes [47,48], endothelial cells [131] and vascular smooth muscle cells. This effect is mediated through the AT1 receptor (AngII type 1 receptor) [33]. In line with this, drugs interfering with the angiotensin system such as ACEIs (angiotensin-converting enzyme inhibitors) down-regulate endotoxin-induced TF activity in monocytes [132]. Recently, it has been shown that also short-term changes in blood pressure result in alterations of plasma TF [133].

**TF and smoking**

Smoking is an independent risk factor for atherosclerosis and atherothrombotic events. ApoE-deficient mice exposed to cigarette smoke had significantly increased TF expression and activity in atherosclerotic plaques [134]. Similarly, in plaques from human carotid arteries obtained from smokers, TF expression was enhanced compared with non-smokers; furthermore, circulating TF activity was elevated in active smokers [121]. Although nicotine is known to induce TF expression in both endothelial and vascular smooth muscle cells in culture [135], the mechanisms by which smoking enhances TF expression in vivo are not fully understood and need to be elucidated further in detail.

**TF and atherosclerosis**

Inflammation plays a key role in the development of atherosclerosis [58,136]. Monocyte infiltration in the intima, followed by transformation into macrophages and foam cells, represent important early steps in atherosclerotic lesion formation [58]. Elevated levels of inflammatory cytokines known to induce TF expression, such as TNF-α and the ILs, are observed in such lesions. In healthy arteries, TF is expressed in fibroblasts and pericytes of the adventitia and, to a lesser extent, in medial smooth muscle cells, whereas virtually no TF antigen is detectable in the intima including endothelial cells [27,137]. In the early stages of atherogenesis, TF mRNA and antigen is expressed in plaque macrophages [27]; at later stages, TF is also detectable in other cells such as foam cells, endothelial cells and smooth muscle cells [27,138]. Under such conditions, TF is found in the extracellular space surrounding cholesterol clefts and within the necrotic core [27,139], where it is
predominantly associated with MPs originating from foam cells, monocytes and lymphocytes [138,140]. Indeed, such MPs represent the major part of TF activity in atherosclerotic lesions [140]. Consistent with these observations, patients with acute coronary syndromes have higher levels of TF antigen in their atherosclerotic plaques compared with patients with stable angina [141].

Apart from the evidence that TF is up-regulated in atherosclerotic lesions, its role in the pathogenesis of atherosclerosis is still controversial. Migration of vascular smooth muscle cells from the media to the intima, followed by proliferation within the neointima, are important remodelling processes after vascular injury and in the formation of atherosclerotic lesions [142]. The TF–FVIIa complex is a stimulus for smooth muscle cell migration in vitro and in vivo; indeed, overexpression of TFPI inhibits this effect [143,144]. Hence TF may contribute to plaque progression and vascular remodelling. Moreover, newly formed microvessels within atherosclerotic plaques can lead to plaque growth and destabilization by promoting inflammation and haemorrhage, and TF, which is importantly involved in angiogenesis [95], may play a role in plaque neovascularization. Nevertheless, the development of atherosclerosis in heterozygous TF-knockout mice, which have a 50 % reduction in vascular TF activity, does not differ from that in control animals; moreover, a 90 % reduction in haematopoietic-cell-derived TF does not alter the development of atherosclerotic lesions either [145]. Thus TF does not appear to influence atherogenesis in mice under these conditions. The question arises, however, of whether a 50 % reduction in overall TF activity or a 90 % reduction in TF activity from bone-marrow-derived cells respectively, is sufficient to modulate atherogenesis in this model or whether the role of TF in atherogenesis should also be tested in a conditional knockout mouse approach targeting the role of TF in vascular smooth muscle cells in particular.

**TF in acute coronary syndromes**

In patients presenting with unstable angina or myocardial infarction, higher levels of TF antigen as well as activity are detected in coronary atherectomy specimens compared with those from patients with stable angina [141,146]. Furthermore, expression of TF in plaque macrophages is elevated in atherectomy specimens from patients with unstable angina and myocardial infarction [147]. In line with this, plasma TF levels are enhanced in patients with acute coronary syndromes compared with controls [148,149]. Moreover, patients with unstable angina or non-STEMI (ST-elevation myocardial infarction) and a high TIMI score (≥4) have higher TF plasma levels than those with a low TIMI score (<3) [150], and an enhanced TF level has been recognized as an important predictor of unfavourable outcome [151]. Monocytes and platelets represent an important source of circulating TF in acute coronary syndromes [152], but MP-associated TF shed from either activated or apoptotic cells may contribute as well, since increased amounts of shed MPs have been observed in patients with endothelial injury [63]. Finally, plaque rupture may contribute further to the elevated levels of TF, as this event leads to the exposure of highly procoagulant plaque content to the circulation [140].

**THERAPEUTIC STRATEGIES**

Arterial thrombus formation results from the interaction of various factors, including the vessel wall, coagulation cascade and platelets. Hence a wide spectrum of drugs interfering with this process is in clinical use. In addition to the classical antithrombotic agents, drugs targeting TF and the TF–FVIIa complex, and thereby interfering with the first steps in the coagulation cascade, have been introduced. Even though different approaches to inhibit TF initially looked promising, their efficacy in inhibiting TF expression and thrombosis in humans has not yet been examined successfully, and further investigations are necessary.

**Inhibitory targets**

**Inhibition of TF synthesis**

TF protein expression may be targeted at either the transcriptional or the post-transcriptional level. Various agents interfere with TF mRNA expression in vitro and in vivo. Hairpin ribozymes destroy TF mRNA and decrease TF induction in cultured vascular smooth muscle cells [153]. Antisense oligonucleotides prevent TF translation by hybridizing to their complementary target mRNA in monocytes [154]. Furthermore, cardiac glycosides such as digoxin inhibit TF translation by lowering intracellular potassium concentrations via impairing Na+/K+-ATPase activity in vitro [155]. Moreover, amiodarone, a widely used anti-arrhythmic drug, impairs TF expression in cultured human vascular cells at the translational level and inhibits, at clinically relevant plasma and tissue concentrations, TF activity and arterial thrombus formation in vivo under real-flow conditions [31]. Similarly, DMSO, used for preservation of haematopoietic progenitor cells, inhibits arterial thrombus formation by impairing TF activity [32].

**Inhibition of TF action**

Major efforts have been undertaken to interfere with the actions of TF, mainly by the use of antibodies. In different thrombosis models, monoclonal anti-TF antibodies reduce arterial thrombus formation [156,157]; in addition, they also shorten lysis time and reduce re-occlusion rates.
following tPA (tissue plasminogen activator) administration [158]. Consistent with this, an anti-TF antibody markedly reduces plaque thrombogenicity in humans [159]. Application of a mutant form of TF is an alternative approach to inhibiting thrombosis. This form of TF, despite binding FVIIa, has reduced catalytic activity [160], and is able to impair arterial thrombosis in a rabbit and a guinea-pig model of arterial thrombosis [160,161].

Specific inhibitory molecules

rTFPI (recombinant TFPI)
The balance between TF and TFPI, the physiological antagonist of TF, is critical for thrombus formation [162]. Administration of rTFPI in humans significantly reduces fibrinogen and platelet deposition [159,163]; additionally, rTFPI inhibits thrombus formation at the site of balloon-induced arterial injury [164]. In addition to systemic administration of rTFPI, local gene transfer by a vector encoding human rTFPI represents an alternative approach. Indeed, overexpression of TFPI in a porcine thrombosis model increased resistance to thrombus formation [165] and reduced intimal hyperplasia [166]. Nevertheless, application of rTFPI may be limited due to induction of apoptosis in cultured endothelial [167] and vascular smooth muscle cells [168], which may contribute to instability of atherosclerotic plaques.

NAPc2 (nematode anticoagulant protein c2)
rNAPc2 (recombinant NAPc2) is an 85-amino-acid protein originally isolated from the haematophagous hookworm *Ancylostoma caninum*. rNAPc2 binds to FX or FXa prior to the formation of the final ternary inhibitory complex with TF–FVIIa [169]. In a double blind placebo-controlled human study, rNAPc2 was found to be safe and well-tolerated at doses ranging from 0.3 to 5 μg/kg of body weight in preventing thrombin generation during coronary angioplasty in combination with aspirin, clopidogrel and heparin [170]. In a phase II clinical trial, however, administration of higher concentrations of rNAPc2 (≥7.5 μg/kg of body weight) was associated with major bleeding [171]. As rNAPc2 is a non-human protein, immunogenic responses after administration are possible; indeed, anti-rNAPc2 IgG were observed in one subject in a cohort of 20 patients receiving rNAPc2, but these IgGs were not biologically active and were not associated with a poorer outcome.

SUMMARY AND OUTLOOK

TF, a transmembrane glycoprotein, is the key trigger of the coagulation cascade; in addition, it participates in regulating different cellular responses. Cytokines, growth factors and biogenic amines induce TF expression in endothelial cells, vascular smooth muscle cells and monocytes. More recently, TF has also been detected in platelets, eosinophils and MPs. Signal transduction mechanisms specific for both the cell type and the stimulus involved regulate TF induction and its cellular distribution. In addition to cellular TF, an important role for blood-borne TF is emerging. At present, however, the relative contribution of vessel-wall-associated compared with blood-borne TF to both thrombus formation and propagation is still debated.

Cardiovascular risk factors, including hypertension, diabetes, dyslipidaemia and smoking, exert elevated plasma levels of TF, which may contribute to the pro-atherosclerotic effect of such risk factors. Moreover, TF expression is up-regulated in atherosclerotic plaques, and large amounts of TF are released into the bloodstream during plaque rupture, leading to acute thrombus formation in patients with unstable angina or acute coronary syndromes. As TF is able to stimulate vascular smooth muscle cell migration and proliferation, it may promote neointima formation not only by initiating thrombosis, but also by direct actions on vascular cells.

Several systemic strategies have been developed for targeting the action of TF. Despite promising pre-clinical results, most of these systemic substances are not yet used in daily clinical practice. In contrast, targeting TF locally, for example with drug-eluting stents, may offer a valid therapeutic option achieving local inhibition of thrombosis without systemic bleeding complications. Therefore increasing efforts should be undertaken to develop such applications.

FUNDING

The authors’ work was supported by the Swiss National Science Foundation [grant number 3200BO-113328/1 (to F.C.T.)); the Bonizzi-Theler Foundation; the Velux Science Foundation [grant number 3200B0-113328/1]; the Wolfermann Nägeli Foundation; the MERCATOR Foundation; and the Swiss Heart Foundation.

REFERENCES


Tissue factor: beyond coagulation in the cardiovascular system


119 Felmelen, D. C., Spencer, C. G., Chung, N. A., Belgore, F. M., Blann, A. D., Bevers, D. G. and Lip, G. Y. (2003) Relation of thrombogenesis in systemic hypertension to angiogenesis and endothelial damage/dysfunction (a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]). Am. J. Cardiol. 92, 400–405


Received 28 November 2008/25 June 2009; accepted 25 June 2009
Published on the Internet 26 October 2009, doi:10.1042/CS20080622