Tissue factor in cardiovascular disease pathophysiology and pharmacological intervention

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Tissue Factor in Cardiovascular Disease:

Pathophysiology and Pharmacological Intervention

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Abstract

Tissue factor (TF), also known as thromboplastin or coagulation factor III, is the major trigger of the coagulation cascade and thereby crucially involved in the maintenance of vascular hemostasis. By binding factor VIIa, the resulting TF:VIIa complex activates the coagulation factors IX and X ultimately leading to fibrin and clot formation. In the vessel wall, TF expression and activity is detectable in vascular smooth muscle cells and fibroblasts and, at a much lower level, in endothelial cells, and can be induced by various stimuli including cytokines, biogenic amines, or growth factors. In addition, TF is found in the bloodstream in circulating cells such as monocytes or leukocytes, in TF containing microparticles, and as a soluble spicing isoform. Besides its well known extracellular role as a trigger of coagulation, TF also functions as a transmembrane receptor, and TF dependent intracellular signalling events regulate the expression of genes involved in cellular responses such as proliferation and migration. TF indeed appears to be involved in the pathogenesis of neointima formation and tumor growth, and increased levels of TF have been detected in patients with cardiovascular risk factors or coronary artery disease as well as in those with cancer. Therefore, pharmacological or genetic inhibition of TF may be an attractive target for the treatment of cardiovascular disease and cancer. Different strategies for inhibition of TF have been developed such as inhibition of TF expression and blockade of TF action. Clinical applications of such strategies need to be tested in appropriate trials, in particular for evaluating the advantages of targeted versus systemic delivery of the inhibitors.

1. Tissue factor: structure and function

1.1. TF protein structure

The TF (thromboplastin, CD142) gene located on chromosome 1 at p21-22 spans a 12.4 kb region and contains six exons encoding a single transmembrane polypetide chain composed of 263 amino acids (Kao et al., 1988; Mackman et al., 1989; Morrissey et al., 1989; Scarpati et al., 1987). The TF protein is divided into three domains: an extracellular (219 amino acids), a membrane-spanning (23 amino acids) and a cytoplasmatic (21 amino acids) region (Breitenstein et al., 2010). TF is considered a class I integral membrane protein since the amino-terminus of the protein is located extracellularly while the cytoplasmatic tail contains the carboxy-terminus. Mobility studies on sodium dodecyl sulfate gels reveal that the molecular weight of the fully glycosylated protein is about 45'000 kDa (Broze et al., 1985). The extracellular domain provides two high affinity sites for the binding of factor VII to TF. This binding is crucial for induction of the conformal changes required to induce the catalytic activity of factor VII (Edgington et al., 1991; Rehemtulla et al., 1991). In addition, anchoring of the TF protein to the membrane via the hydrophobic transmembrane domain has been demonstrated to be essential for procoagulant activity of the TF protein. However, the nature of the anchoring domain does not seem to play a role (Paborsky et al., 1991). Indeed, deletion of the cytoplasmtic tail is not paralleled by an impaired TF procoagulant activity (Paborsky et al., 1991). Therefore, recent studies focussed on possible non-hemostatic functions of this domain. Experiments performed mainly in fibroblasts and cancer cell lines revealed that phosphorylation of the cytoplasmatic tail activates intracellular signaling

pathways and induces cellular responses which are described in the following chapter.

In 2003, an alternatively spliced (as) isoform of TF mRNA lacking the fifth exon and exhibiting a frameshift mutation in the carboxy-terminal region was characterized (Bogdanov et al., 2003). This TF protein lacks membrane anchorage and is detectable in the plasma as a soluble TF protein. While the asTF protein has been described in different species and cell types, its contribution to the TF procoagulant activity in plasma as well as thrombus formation in vivo remains barely understood (Bogdanov et al., 2006; Mackman, 2007).

1.2. Tissue factor: key trigger of coagulation

In intact vessels, blood circulates as a fluid due to the physiological balance between natural procoagulant and anticoagulant factors. The clotting cascade defines a complex system of zymogens and enzymatic reactions designed to limit blood loss and ultimately restore vascular integrity by formation of a clot following vessel injury. Initially, initiation of coagulation was thought to be triggered by two distinct pathways: the intrinsic, or contact activation pathway and the extrinsic, or tissue factor (TF) pathway. Even though the two pathways are independently activated, the distinction between both of them has been blurred since they share a final common pathway and, in addition, there is crosstalk between the two systems. The intrinsic pathway is activated when blood or plasma comes in contact with subendothelial connective tissue or negatively charged surfaces and involves circulating factors such as factor XII, factor XI, factor IX, prekallikrein, and high molecular weight kininogen (Gailani and Renne, 2007). However, in has been questioned that this pathway is required for maintenance of normal haemostasis, since congenital deficiency in the involved

factors, for example factor XII, does not result in an increased bleeding tendency or clinically appearant pathologies in humans.

Coagulation through the TF pathway is activated when blood interacts with a TF expressing cell surface, such as it occurs after endothelial erosion, plaque rupture, or other vascular injury (Fig. 1). The initial step consists in the reversible binding of the zymogen factor VII (FVII) or the activated FVII (FVIIa) to membrane-bound TF (Mackman, 2009; Mackman et al., 2007), resulting in the formation of both TF:FVII and TF:VIIa complexes. Only the latter, however, possesses the enzymatic activity required for further activation of downstream proteases. Once bound to TF, the inactive FVII is converted to FVIIa via limited proteolysis by several plasma proteases, such as XIIa, Xa, Ixa, or thrombin, generating a positive feedback loop (Steffel et al., 2006). The TF:FVIIa complex is indeed a potent activator of the coagulation cascade and is composed of TF acting as a cofactor with regulatory functions, and the serine protease FVIIa exerting catalytic functions (Breitenstein et al., 2010). The TF:VIIa complex activates factor IX to factor IXa which in association with VIIIa catalyzes the conversion of factor X to Xa. In addition, factor X is directly converted to factor Xa by the TF:FVIIa complex. Factor Xa in association with factor Va and divalent calcium forms the so called prothrombinase complex which cleaves prothrombin to form thrombin (Breitenstein et al., 2010). Thrombin exerts direct effects on coagulation through conversion of fibrinogen to fibrin, activation of platelets, and activation of various proteases of the coagulation cascade such as FVII, FXI, and FV representing an important auto-feedback loop. Besides its well known role in clot formation, the functions of thrombin extend from coagulation to vascular remodelling by stimulating proliferation of vascular smooth muscle and endothelial cells as well as secretion of growth factors ultimately leading to

restoration of vascular integrity (Ferrara and Davis-Smyth, 1997; Maragoudakis et al., 2002; Tsopanoglou et al., 2002; Tsopanoglou et al., 2004).

1.3. Tissue factor: a signaling receptor

The tight link between hemostasis and progression of vascular alterations such as inflammation or atherosclerosis provides evidence for complex interactions between the coagulation cascade and vascular pathophysiology. Mainly because of its similarity to class II cytokine receptors recent studies suggested a non-hemostatic role of TF in different biological processes through induction of intracellular signaling events (Bazan, 1990). The following chapter provides an overview of TF mediated cell signaling (Fig. 1).

Evidence for TF:VIIa induced signaling is derived from experiments in which FVIIa binding to TF was shown to induce intracellular calcium transients in different types of TF expressing cells (Camerer et al., 1996; Rottingen et al., 1995). This observation was followed by studies demonstrating that binding of factor VIIa to TF induces the activation of the three major mitogen activated protein (MAP) kinase family members p38 MAP kinase, p42/p44 MAP kinase, and c-Jun N-terminal kinase (JNK) (Camerer et al., 1999; Poulsen, et al., 1998). In fibroblasts, the TF-FVIIa induced activation of Src-like kinases and PI3-kinase leads to activation of the downstream Rho-like guanosine triphosphatases Rac and Cdc42 resulting in cytoskeletal reorganization (Versteeg et al., 2000).

Hence, by activating different kinases involved in signal transduction, the TF:VIIa complex regulates the transcription of numerous genes involved in physiological and pathophysiological processes such as cell migration, cell growth, or apoptosis. Indeed, binding of factor VIIa to TF induces activation of key transcription factors

such as Egr-1 or nuclear factor kappa B and the activation of the RNA polymerase A (Camerer, et al., 1999; Pendurthi et al., 1997). Moreover, microarray analysis of the gene expression profiles revealed increased mRNA levels of growth factors (i.e. connective tissue growth factors CCN1 and CCN2, fibroblast growth factor 5) and inflammatory cytokines (i.e. interleukin-1β, interleukin-8) following factor VIIa and TF interaction (Camerer et al.,2000; Pendurthi, et al., 2000).

Interestingly, induction of signal transduction events was demonstrated to depend on the catalytic activity of factor VIIa, because inactivated factor VII or anti-TF antibodies blunt these effects (Camerer et al., 1999; Camerer et al., 1996; Pendurthi et al, 2000; Poulsen et al., 1998). Consistent with this observation, the TF:VIIa complex initiates intracellular signalling via G-protein-coupled protease-activated receptors (PARs) (Riewald & Ruf, 2002; Siegbahn, 2000). Currently, 4 different PARs have been characterized: PAR1, PAR2, PAR3, and PAR4. There is growing evidence that TF:VIIa signaling is mediated mainly by PAR2 and to a lesser extent by PAR1, while PAR3 and PAR4 do not seem to be involved. For instance, in fibroblasts derived from PAR1 knockout mice, the FVIIa induced response only occurred when TF and PAR2 where co-expressed (Camerer et al., 2000). Moreover, data obtained in a carcinoma cell line demonstrate that antibodies targeting PAR2, but not PAR1, blunt TF:VIIa induced IL-8 expression and smooth muscle cell migration (Marutsuka et al., 2002).

Even though not essential for TF:VIIa induced activation of MAP kinases and gene expression, the cytoplasmic domain of TF is implicated in cytoskeletal organization and cell migration (Bromberg, Konigsberg, Madison, Pawashe, & Garen, 1995; Mueller & Ruf, 1998). Protein kinase C mediated phosphorylation of the cytoplasmatic domain at the serine residues Ser253 and Ser258 enables the interaction of the cytoplasmatic tail with the actin-binding protein 280 thereby regulating actin filament rearrangement (Ott et al., 1998; Zioncheck et al., 1992).

Studies performed in mice lacking the cytoplasmatic tail of TF demonstrated the crucial role of the cytoplasmatic domain for vascular smooth muscle migration and vascular remodelling after injury in vivo (Ott et al., 2005).

Since increasing evidence suggests an essential role of TF as an important signal transducer beyond its well-known functions in coagulation, developing specific inhibitors that suppress either TF:VIIa mediated signaling or its coagulant function may offer interesting therapeutic approaches for the treatment of TF associated diseases.

2. Tissue factor in cardiovascular disease

2.1. TF distribution

2.1.1. Vascular TF

Under normal hemostatic conditions, TF is only barely expressed in endothelial cells (Fig. 2). Inflammatory cytokines such as tumour necrosis factor-α (TNF-α) (Holy et al., 2005) or interleukin-1β (Napoleone et al., 1997) enhance TF expression. This induction of TF protein is mainly mediated by the mitogen-activated protein (MAP) kinases p38, extracellular-signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) which activate transcription factors such as nuclear factor kappa B, activator protein-1, or early growth-response gene product-1, all of them involved in transcription of the TF gene (Bavendiek et al., 2002; Mackman, 1997; Mechtcheriakova et al., 2001). Depending on the stimulus, induction of TF expression occurs via activation of either all three MAP kinases (Holy et al., 2009; Steffel et al., 2006; Steffel et al., 2005) or only a single pathway (Mechtcheriakova et al., 2001). Protein kinase C as well as the Rho-kinase pathway have also been shown to mediate the induction of endothelial TF expression (Eto et al., 2002; Mechtcheriakova et al., 2001; Zhang et al., 2007). Whereas the MAP kinase, Rho-kinase, and protein kinase C pathways regulate TF expression in a positive manner, the phosphoinositide 3 kinase pathway exerts a negative regulatory effect on TF expression (Blum et al., 2001; Steffel et al., 2005). Indeed, downstream targets of phosphoinositide 3 kinase are able to regulate TF expression at both the transcriptional and the translational level. Akt and glycogen synthase kinase 3β regulate TF transcription (Eto et al., 2005), whereas the mammalian target of rapamycin and p70S6 kinase inhibit TF translation (Guba et al., 2005; Steffel et al.,

2005). An additional mechanism affecting the regulation of TF protein expression is stabilization of TF messenger RNA. A recent study in endothelial cells demonstrated that a decrease in TF promoter activity may be counteracted by an increased TF mRNA stability leading to an overall enhanced TF protein expression (Holy et al., 2009).

In contrast to endothelial cells, vascular smooth muscle cells constitutively express TF providing a hemostatic barrier after vascular injury (Fig. 2; Breitenstein et al., 2008; Camici et al., 2006; Schecter et al., 1997; Wilcox et al., 1989). Similar to endothelial cells, TF expression in vascular smooth muscle cells is enhanced by several receptor ligands such as CD40 ligand, histamine, thrombin, bacterial endotoxin, oxidized LDL, or C-reactive protein (Camici et al., 2006; Kamimura et al., 2004; Llorente-Cortes et al., 2004; Taubman et al., 1993; Wu et al., 2008). Even though not investigated to the same extent as in endothelial cells, the MAP kinase and phosphoinositide 3-kinase pathways regulate TF expression also in vascular smooth muscle cells.

2.1.2. Blood-borne TF

More recently, TF has been detected at low levels in plasma and urine, and these levels increase in several inflammatory disease conditions such as atherosclerosis, disseminated intravascular coagulation, glomerulonephritis, sepsis, or diabetes.

Monocytes are thought to represent a major source of the plasmatic TF pool. Monocytes constitutively express TF and its expression is enhanced after exposure to numerous inflammatory mediators (Cai et al., 2007; Cermak et al., 1993; Guha & Mackman, 2002; Lewis et al., 1995; Luyendyk et al., 2008; Mach et al., 1997). Indeed, elevated levels of TF have been measured in plasma during septic states and may account for the thrombotic complications encountered in those patients

(Osterud & Bjorklid, 2001; ten Cate et al., 2000). Endotoxin-induced TF expression is mediated via MAP kinases (Guha & Mackman, 2002; Lewis et al., 1995) and the consecutive activation of the transcription factors EGR-1, c-Fos/c-Jun, and nuclear factor kappa B. In addition, endotoxin also increases TF mRNA stability (Brand et al., 1991).

TF has also been described in circulating granulocytes. Eosinophils, neutrophils, and basophils have been found to express TF (Kambas et al., 2008; Maugeri, Brambilla et al., 2006; Maugeri et al., 2006; Moosbauer et al., 2007; Muller et al., 2003). Both the trancription and the translocation of TF to the cell surface are enhanced upon stimulation of these cells with granulocyte/macrophage colony-stimulating factor or platelet-activating factor (Moosbauer et al., 2007). These findings may explain the pro-thrombotic state associated with hypereosinophilia (Cugno et al., 2009).

Even though TF-containing platelets have been described by several groups (Falati et al., 2003; Muller et al., 2003; Panes et al., 2007; Perez-Pujol et al., 2005; Zillmann et al., 2001) the role of platelet derived TF remains debatable (Butenas et al., 2005). In platelets, TF is encountered in different compartments including the membrane, the matrix of α-granules, and the canalicular system; it is translocated to the cell surface upon activation by different agonists. Since TF messenger RNA is not found in human megakaryocytes, the source of platelet derived TF remains a matter of debate. One hypothesis suggests that TF containing microparticles derived from activated monocytes, leucocytes (Rauch et al., 2000), or endothelial cells (Aras et al., 2004; Camera et al., 2003) fuse with the platelet membrane through a receptor mediated process (Falati et al., 2003; Rauch et al., 2000) and thereby deliver TF into platelets. However, other studies demonstrated the presence of a spliced TF mRNA in activated human platelets confirming an increased TF protein synthesis and activity in ADP stimulated platelets (Camera et al., 2003).

TF containing microparticles have been well characterized in plasma. Besides endothelial and vascular smooth muscle cells (Abid Hussein et al., 2003; Lechner et al., 2007; Moosbauer et al., 2007; Schecter et al., 2000), monocytes and platelets are the main source of TF containing microparticles (Hron et al., 2007; Siddiqui et al., 2002). Despite studies demonstrating the role of microparticle derived TF in the thrombin generation *in vitro* (Sturk-Maquelin et al., 2003), doubts remain about its contribution to thrombus formation *in vivo* (Biro et al., 2003; Day et al., 2005).

The full-length form of TF mRNA contains 6 exons and splicing of exon 5 results in a frame-shift mutation generating a soluble alternatively spliced TF protein lacking the transmembrane domain (Bogdanov et al., 2003). Apart from plasma, alternatively spliced TF has been found in several tissues and cell types including the lung, placenta, vascular smooth muscle cells, and keratinocytes. Whereas it remains unclear whether alternatively spliced TF exerts pro-coagulant activity (Szotowski et al., 2005) more recent reports have revealed a role of alternatively spliced TF in angiogenesis by promoting endothelial cell migration and differentiation (Censarek et al., 2007; Y. He et al., 2008).

2.1.3. Cardiac TF

In contrast to skeletal myocytes, cardiac myocytes express the full length form of TF under basal conditions (Hartzell et al., 1989; Mackman et al., 1993). Studies performed in mice expressing low levels of TF revealed a potential role of TF in the maintenance of cardiac hemostasis, since these mice had increased cardiac hemorrhages and hemosiderin deposition ultimately leading to cardiac fibrosis and a reduction in left ventricular function as compared to wild type animals (Pawlinski et al., 2002). Phagocytosis of hematin derived from hemorrhaging erythrocytes is thought to play a key role in this process. Interestingly, mice lacking factor IX had

normal hearts, underlining the specific role of the TF:VIIa complex in hemostatic protection of the heart.

2.2. TF in cardiovascular disease

2.2.1. TF in thrombus formation

Although TF is critically involved in initiation of the coagulation cascade, questions regarding the role and contribution of the different TF pools in arterial thrombus formation remain open.

Under normal hemostatic conditions, TF is constitutively expressed in the vascular smooth muscle layer of the media and in fibroblasts present in the vessels as well as in the adventitia surrounding the vessels (Drake et al., 1989; Fleck et al., 1990). Hence, disruption of blood vessel integrity promotes the interaction between circulating FVII and the vascular TF pool leading to initiation of the clotting cascade (Fig. 2). Moreover, the release of inflammatory triggers following vascular injury or plaque rupture induces the expression of TF in endothelial cells as well as in circulating cells thereby potentiating a full coagulation response (Steffel et al., 2006). In contrast to the initiation phase of coagulation, the mechanisms promoting the propagation of clot growth are not as evident yet.

Blood-borne TF may contribute to the propagation of the clotting reaction. This consideration is based on the fact that the maintenance of coagulation requires a constant activity of TF as a cofactor and that the formed fibrin clot isolates and prevents the initially formed TF:VIIa complexes from reacting with circulating new inactive coagulation factors (Hathcock & Nemerson, 2004). This hypothesis is supported by the observation that TF is detected at very low levels in the blood unable to trigger a coagulant response whereas the TF concentration reaches functionally significant levels on the surface of a forming thrombus (Bogdanov et al.,

2003; Chou et al., 2004; Jesty & Beltrami, 2005). In addition, TF has been found embedded in murine (Day et al., 2005; Hoffman et al., 2006) and human (Wysokinski et al., 2004) thrombi. The role of blood-borne TF in coagulation is strengthened by an in vitro study demonstrating that the sub-picomolar TF concentrations found in blood cause an increase in thrombin formation under flow, but not under no-flow conditions (Okorie et al., 2008).

On the other hand, recent experiments performed in mathematical as well as whole blood models revealed different stages of TF requirement in the clotting process and showed that the propagation of coagulation is TF independent once thrombin has started to be formed (Orfeo et al., 2005). By abrogating TF activity with specific antibodies targeting either VIIa or TF, 3 phases in the clotting process could be distinguished: a first short (<10 seconds) period of absolute TF:VIIa dependence; a second period (10-240 seconds) of partial TF:VIIa dependence that decreases with the progress of the reaction, and a period which is TF:VIIa independent beginning after 2 minutes. In addition, this study revealed that the initial activation of procoagulant proteases by the TF:VIIa complex is sufficient to maintain a pool of activated procoagulant catalysts able to maintain a sustained coagulation response in a TF independent manner.

Since venous thrombosis is not always associated with vessel damage, circulating TF may play an important role in this pathology. In a model of jugular vein thrombosis inhibition of TF reduced thrombus growth in the uninjured vein (Himber et al., 2003). However, in another study, hematopoietic cell-derived TF did not affect venous thrombosis in an inferior vena cava thrombosis model in the mouse (Day et al., 2005). Hence, the exact role of the different TF pools and their contributions to the propagation of clot formation remains controversial and further studies are definitely required.

2.2.2. TF in inflammation and atherosclerosis

Inflammation is tightly linked to the development of atherosclerosis (Hansson, 2005). Elevated levels of inflammatory cytokines known to induce TF expression such as TNF-α and interleukins are observed in early atherosclerotic lesions. In the early stages of atherogenesis, TF mRNA and antigen is expressed in macrophages (Thiruvikraman et al., 1996; Wilcox et al., 1989), and as the lesions progress, TF is increasingly expressed in other cell types such as endothelial cells and smooth muscle cells (Fig. 2). Within the necrotic core (Marmur et al., 1996), it is predominantly associated with TF containing microparticles released by foam cells, monocytes, lymphocytes, and smooth muscle cells; in addition, it is detected in the extracellular space (Mallat et al., 2000). During plaque rupture, the content of the core comes in contact with the circulating blood and TF present within the plaque as well as expressed by activated endothelial and smooth muscle cells triggers thrombus formation leading to an acute vascular event. Indeed, higher levels of TF are measured in atherosclerotic plaques of patients suffering from an acute coronary syndrome as compared to patients exhibiting stable angina (Annex et al., 1995).

Recent studies implicate that TF and TF:VIIa signaling may also play a role in the pathogenesis of atherosclerosis. The TF:VIIa complex has been shown to be a strong chemotactic stimulus for vascular smooth muscle cells (Sato et al., 1996). Inhibition of TF:VIIa by overexpression of tissue factor pathway inhibitor leads to a consecutive reduction of vascular smooth muscle cell migration and impairs vascular remodeling in the mouse in vivo (Sato et al., 1999; Singh et al., 2001). Since TF:VIIa mediated cell signalling induces a number of gene products involved in cell proliferation and migration, it is very likely that similar effects occur in TF expressing cells within atherosclerotic lesions. TF:VIIa signalling indeed enhances the expression of the

connective tissue growth factors CCN1 and CCN2 in fibroblasts and vascular smooth muscle cells. Similar to TF, CCN1 and CCN2 mRNA levels are highly expressed in atherosclerotic lesions of apoE-/- mice and in human atherosclerotic plaques (Oemar & Luscher, 1997; Oemar et al., 1997). Hence, it is conceivable that by inducing the expression of CCN1 and CCN2 and possibly other mediators as well, TF:VIIa promotes plaque progression via enhanced extracellular matrix accumulation and vascular smooth muscle cell proliferation. In addition, a role of CCN1 and CCN2 in monocyte and platelet adhesion to the subendothelial matrix after endothelial injury has also been described (Jedsadayanmata et al., 1999; Schober et al., 2002).

In response to inflammatory mediators, TF transcription can be induced in different cell types. On the other hand, TF expressed on these cells appears to regulate the inflammatory response. During sepsis, the TF pathway induces a lethal inflammatory exacerbation independent of thrombin generation and clot formation (Creasey et al., 1993; Taylor et al., 1991). Inhibition of the TF:VIIa complex is paralleled by a decrease in interleukin secretion and a reduction in inflammatory infiltrations in several tissue during sepsis (Miller et al., 2002). This finding is confirmed by the observation that mice suffering from sepsis and expressing low TF levels display reduced interleukin levels and an increased survival compared with control mice (Pawlinski et al., 2004). Studies performed in PAR2 deficient mice suggest that the pro-inflammatory TF:VIIa signaling response is likely mediated via PAR2 (Pawlinski et al., 2004). However it is worth noting that deficiency in PAR1 or PAR2 alone has no effect and that a reduction in inflammation and mortality is only observed in the context of a combined inhibition of thrombin generation and PAR2 receptor, indicating a tight link between coagulation and inflammation.

2.2.3. TF in acute coronary syndromes

High levels of TF have been detected in coronary atherectomy specimens from patients with unstable angina or myocardial infarction (Ardissino et al., 1997). In line with this, plasma TF levels are elevated in patients with acute coronary syndromes compared with controls (Misumi et al., 1998; Soejima et al., 1996) and correlate with an unfavourable outcome in these patients (Soejima et al., 1999). In addition, patients with unstable angina or non-ST-elevation myocardial infarction and a high TIMI score (≥4) exhibit increased TF plasma levels as compared to those with a low TIMI score (<3) (Lee et al., 2005).

Percutaneous transcoronary angioplasty and coronary stent implantation has become the gold standard in the treatment of culprit lesions in acute coronary syndromes. The local inflammatory reaction following balloon dilatation and stent implantation (Brunetti et al., 2007) induces the release of TF from the dissected plaque. Thus, elevated levels of TF positively correlate with the incidence of restenosis after percutaneous interventions, presumably due to promigratory effects of TF on vascular smooth muscle cells (Tutar et al., 2003). Despite the reduction in restenosis following deployment of drug eluting stents (DES) when compared to bare metal stents, stent thombosis rates have not been decreased with the use of DES and may even be higher (Iakovou et al., 2005). One possible explanation may be that the drugs eluting from the stents which are intended to inhibit restenosis by reduction of vascular smooth muscle cell migration and proliferation exert unwanted biological effects on these or other cell types in the vasculature, in particular endothelial cells. The drugs applied for coating the first and second generation stents, like rapamycin, everolimus, or zotarolimus, enhance TF expression in vascular cells and accelerate arterial thrombus formation via inhibition of the mTOR/p70S6K pathway (Camici et al., 2010; Steffel et al., 2005). Similarly, paclitaxel, another widespread stent coating drug was shown to increase endothelial TF expression and activity through activation

of the MAP kinase JNK (Stahli et al., 2006). Hence, these findings may raise important issues in the context of drug eluting stent design since coating a stent with a drug exerting not only antiproliferative but also antithrombotic properties may offer new options in the interventional treatment of vascular disease.

2.2.4. TF and cardiovascular risk factors

Diabetes mellitus is associated with an increase in plasmatic TF activity (El-Ghoroury et al., 2008), TF antigen levels (Limet al., 2004), and TF containing microparticles (Diamant et al., 2002). In line with these observations, improvement of glycemic control or application of oral antidiabetics such as the peroxisome-proliferator-activated receptor-γ agonist rosiglitazone downregulate TF protein expression (Golledge et al., 2007) in these patients. Consistent with this, high glucose increases TF antigen and activity in monocytes derived from healthy subjects (Stegenga et al., 2006; Vaidyula et al., 2006). A similar effect of high glucose on TF expression was also observed in human endothelial cells (Boeri et al., 1989). In diabetes, hyperglycaemia induces the formation of advanced glycation end-products (AGE), which upregulate TF expression in endothelial cells and monocytes via nuclear factor kappa B (Bierhaus et al., 1997; Bierhaus et al., 1997; Kislinger et al., 2001). Not surprisingly, higher levels of AGE and TF were observed in the vasculature of diabetic mice, and blockade of the receptor for AGE significantly reduced the increased TF expression in the aorta of these mice (Kislinger et al., 2001).

Patients with hyperlipidemia display an increased risk of developing thrombosis (Durrington, 2003). Elevated low density lipoprotein (LDL) levels are associated with an increased TF plasma activity (Sambola et al., 2003). Oxidized LDL triggers TF protein synthesis in monocytes (Wada et al., 1994), endothelial cells (Fei et al., 1993), and vascular smooth muscle cells (Penn et al., 1999). In contrast, high-density

lipoproteins (HDL) reduce the thrombin-induced TF expression in endothelial cells through PI3K pathway activation (Viswambharan et al., 2004). Moreover, 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) reduce TF expression in monocytes (Markle et al., 2003), endothelial cells (Eto et al., 2002), and vascular smooth muscle cells (Brandes et al., 2003). These findings have been confirmed by studies performed in ApoE-/- mice in which simvastatin and rosuvastatin treatment reduced TF antigen expression in atherosclerotic lesions independent of their lipid lowering effects (Bea et al., 2003; Monetti et al., 2007). Fibrates, which are also in use as lipid lowering drugs in the treatment of hyperlipidemia, also exert pleiotropic effects on vascular TF expression beyond their lipid lowering properties (Marx et al., 2001).

In contrast to normotensive subjects, hypertensive patients display increased plasma levels of TF antigen (Felmeden et al., 2003), which can be lowered by an appropriate antihypertensive management (Koh et al., 2004). Several mechanisms are thought to mediate this enhanced TF expression observed in arterial hypertension. For instance, the chronic exposure to shear stress induces the activation of transcription factors and consequently the expression of TF in endothelial cells (Dielis et al., 2005). On the other hand, angiotensin II increases TF expression in monocytes (M. He et al.,, 2006), endothelial cells (Dielis et al., 2005), and vascular smooth muscle cells via the angiotensin II type 1 receptor. Consistent with this observation, ACE inhibitors and angiotensin II type 1 receptor antagonists reduce TF plasma activity in hypertensive patients and inhibit endotoxin-induced TF expression in monocytes (Napoleone et al., 2000).

3. Therapeutic implications

The fate of a forming thrombus is determined by the balance of numerous factors, including vessel wall, coagulation system, and aggregating platelets. In addition to the classical antithrombotic agents, drugs targeting TF and the TF:FVIIa complex have been developed, since targeting the initiation phase of thrombosis may prevent thrombus formation more efficiently. Considering the non-hemostatic properties of the TF:VIIa complex, inhibition of TF may also interfere with the pro-migratory and pro-proliferative effects of TF.

Despite promising experimental studies suggesting an effective reduction in thrombosis with a lower bleeding risk as compared to other coagulation inhibitors such as heparin or direct thrombin inhibitors, approaches to inhibit TF not yet been applied successfully in humans, and further large scale clinical trials as well as novel concepts of delivery are required.

3.1. Inhibition of TF production

Inhibition of TF production can be achieved by genetic and pharmacological intervention at both the transcriptional and the post-transcriptional level (Fig. 3). Hairpin ribozymes degrade TF mRNA and decrease TF induction in vascular smooth muscle cells (Cavusoglu et al., 2002). Antisense oligonucleotides hybridize to the complementary TF mRNA sequence and thereby inhibit translation of the TF protein (Stephens & Rivers, 1997). Dimethyl sulfoxide, used for long-term storage of haematopoietic stem cells, inhibits arterial thrombus formation by impairing TF activity (Camici et al., 2006). Further, cardiac glycosides such as digoxin and ouabain inhibit TF translation by lowering the intracellular potassium concentration through impaired Na^+/K^+-ATPase activity (Stahli et al., 2007). Even more interesting, the

class III anti-arrhythmic drug amiodarone inhibits the translation of the TF protein in human vascular cells and impairs arterial TF expression as well as arterial thrombus formation in mice in vivo (Breitenstein et al., 2008). These pleiotropic effects of amiodarone may at least in part account for the beneficial action of the drug on prognosis of patients with heart failure and coronary artery disease (Breitenstein et al., 2008). More recently, the phytosterole guggulsterone as well as the green tea polyphenol epigallocatechin-3-gallate, at concentrations detected in plasma after oral ingestion, were observed to inhibit TF induction through inhibition of MAP kinase activation in human vascular cells and to reduce TF expression in the carotid artery of mice in vivo (Gebhard et al., 2009; Holy, Stampfli et al., 2009). These studies demonstrate that both synthetic and naturally occurring drugs can inhibit the induction of TF in human vascular cells as well as thrombus formation in vivo. Such effects may at least in part account for the established beneficial actions of the above mentioned drugs on the prognosis of patients with cardiovascular disease. Hence, clinical trials assessing the application of these drugs in the context of TF inhibition and thrombus prevention should be performed. Ideally, the drugs would have to be applied in a targeted local manner in order to achieve a maximal effect in the vessel territory at risk for thrombosis while reducing systemic bleeding complications.

3.2. Anti-TF antibodies

Administration of anti-TF antibodies (Fig. 3) delays thrombus formation in a rabbit carotid thrombosis model and reduces the infarction area following ligation of coronary arteries (Erlich et al., 2000; Ragni et al., 1996). A clinical trial (PROXIMATE-TIMI 27) performed in 26 subjects suffering of stable coronary disease tested the impact of a chimeric monoclonal antibody targeting human TF at the factor X binding site (Sunol-cH36/ALT836) (Morrow et al., 2005). Preliminary results

showed a dose dependent anticoagulant effect with only minor bleeding. Application of Sunol-cH36/ALT836 after endarterectomy in chimpanzees reduced local thrombus formation and improved vessel patency after 30 days without increasing bleeding time or surgical blood loss, indicating that early inhibition of coagulation with TF inhibitory antibodies reduces arterial thrombus formation effectively after vascular injury (Jiao et al., 2010).

Considering the role of TF signalling in tumor angiogenesis, growth, and metastasis, inhibition of TF may also translate into anti-neoplastic effects. Indeed, administration of a monoclonal anti-TF antibody (CNTO 859) reduced breast cancer metastasis and tumor growth in xenograft models (Ngo et al., 2007).

3.3. Recombinant tissue factor pathway inhibitor (rTFPI)

TFPI is the physiological inhibitor of TF activity and the balance between TF and TFPI is critical for thrombus formation (Pedersen et al., 2005). By binding the active site of FXa, TFPI interferes with the activity of the TF:VIIa complex (Fig. 3). Administration of rTFPI inhibits thrombus formation at the site of balloon-induced arterial injury and reduces plaque thrombogenecity in humans (Badimon et al., 1999; Harker et al., 1996; St Pierre et al., 1999). In addition, local adenoviral overexpression of TFPI in arteries increases the resistance to thrombus formation (Nishida et al., 1999) and reduces intimal hyperplasia in injured vessels (Zoldhelyi et al., 2001).

Despite of these positive actions, application of rTFPI may be limited because of its apoptotic effects observed in cultured endothelial and vascular smooth muscle cells, which may contribute to instability of atherosclerotic plaques (Fu et al., 2008; Hamuro et al., 1998). Moreover, a randomized controlled study including 1754 patients with severe sepsis and high international normal ratio (INR) found no effect on all-cause

mortality (Abraham et al., 2003). Therefore, additional trials are needed with patients exhibiting cardiovascular diseases other than severe sepsis and being in a better general condition.

3.4. Nematode anticoagulant protein (NAPc2)

Recombinant nematode anticoagulant protein c2 (rNAPc2), isolated from the saliva of the haematophagous hookworm *Ancylostoma caninum* is a factor Xa dependent small protein inhibitor of the TF:VIIa complex which binds to a site on factor Xa that is distinct from the catalytic centre (exo-site) (Fig. 3; Bergum et al., 2001). In a double blind placebo-controlled trial, rNAPc2 was found to be safe and well-tolerated at doses ranging from 0.3 to 5 µg/kg body weight in preventing thrombin generation during coronary angioplasty in combination with aspirin, clopidogrel, and heparin (Moons et al., 2003). A more recent phase II clinical study (ANTHEM-TIMI-32) demonstrated that in patients with non ST-elevation acute coronary syndrome managed with standard antithrombotics and an early invasive approach, additional proximal inhibition of the coagulation cascade with rNAPc2 was well tolerated and not associated with an increase in major or minor bleedings as compared with a placebo treatment (Fluture et al., 2007; Giugliano et al., 2007). A novel finding in the ANTHEM-TIMI-32 trial was that the doses of rNAPc2 suppressing thrombin generation through 48 h were associated with a ≥50% reduction in ischemia on continuous ECG. This provides a link between the mechanism of action of this drug and the hypothesized benefit in patients with plaque rupture and vessel wall injury. However, the trial was not powered for such an assessment and larger trials are required to confirm the hypothesis that rNAPc2 reduces clinical events. The fact that rNAPc2 has a half-life of 50 to 60 hours and can be administered subcutaneously,

prolonged inhibition of thrombin generation by rNAPc2 for several weeks may have the potential to improve the recommended discharge regimen after ACS. However, immune responses after administration may limite its clinical application, since rNAPc2 is a non-human protein. Anti-rNAPc2 immunglobulins were indeed observed in one subject out of 20 patients receiving rNAPc2. Although these antibodies were not biologically active and without clinical relevance, the fact that they occurred in a high percentage of patients will have to be considered in future trials.

4. Conclusions

Major progress has been achieved in decrypting the biological effects and clinical relevance of TF in the context of cardiovacular disease. TF is a transmembrane glycoprotein, the main trigger of coagulation and, beyond this, an intracellular signal transducer. Several signalling pathways, specific for both the cell type and the stimulus, regulate TF induction and its cellular distribution. The discovery of blood-borne TF has launched a new debate about its in vivo relevance, since the relative contribution of vessel-wall as compared to blood-borne TF for thrombus formation and propagation remains unclear.

Elevated levels of TF activity are present in patients with cardiovascular risk factors and in subjects suffering of coronary artery disease. TF expression is up-regulated in atherosclerotic plaques, and cellular TF as well as extracellular TF contained in microparticles come in contact with the blood during endothelial erosion or plaque rupture. Therefore, TF contributes critically to the development of acute vascular events such as myocardial infarction or stroke. On the other hand, TF may promote the progression of atherosclerosis by enhancing vascular smooth muscle cell migration and proliferation.

Since targeting the initiation phase of the clotting cascade may be more effective in preventing thrombus formation than at later stages, several strategies have been developed to inhibit TF expression or activity. Despite promising experimental results, the clinical benefit of these new approaches in comparison with current antithrombotic regimens remains to be demonstrated in large scale trials. Since the major part of TF activity is cell-associated and induced by vascular inflammation, a targeted local inhibition of TF may well prove to be the most effective approach with a minimal effect on the systemic balance of thrombosis and bleeding.

Figure Legends

Figure 1:

TF exerts extracellular effects as main trigger of coagulation as well as intracellular effects on signal transduction.

Figure 2:

Expression of TF in different cell types during the pathogenesis of atherosclerotic lesions.

Figure 3:

Different approaches for inhibition of TF.

References

Abid Hussein, M. N., Meesters, E. W., Osmanovic, N., Romijn, F. P., Nieuwland, R., & Sturk, A. (2003). Antigenic characterization of endothelial cell-derived microparticles and their detection ex vivo. *J Thromb Haemost, 1*(11), 2434-2443.

Abraham, E., Reinhart, K., Opal, S., Demeyer, I., Doig, C., Rodriguez, A. L., et al. (2003). Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. *JAMA, 290*(2), 238-247.

Annex, B. H., Denning, S. M., Channon, K. M., Sketch, M. H., Jr., Stack, R. S., Morrissey, J. H., et al. (1995). Differential expression of tissue factor protein in directional atherectomy specimens from patients with stable and unstable coronary syndromes. *Circulation, 91*(3), 619-622.

Aras, O., Shet, A., Bach, R. R., Hysjulien, J. L., Slungaard, A., Hebbel, R. P., et al. (2004). Induction of microparticle- and cell-associated intravascular tissue factor in human endotoxemia. *Blood, 103*(12), 4545-4553.

Ardissino, D., Merlini, P. A., Ariens, R., Coppola, R., Bramucci, E., & Mannucci, P. M. (1997). Tissue-factor antigen and activity in human coronary atherosclerotic plaques. *Lancet, 349*(9054), 769-771.

Badimon, J. J., Lettino, M., Toschi, V., Fuster, V., Berrozpe, M., Chesebro, J. H., et al. (1999). Local inhibition of tissue factor reduces the thrombogenicity of disrupted human atherosclerotic plaques: effects of tissue factor pathway inhibitor on plaque thrombogenicity under flow conditions. *Circulation, 99*(14), 1780-1787.

Bavendiek, U., Libby, P., Kilbride, M., Reynolds, R., Mackman, N., & Schonbeck, U. (2002). Induction of tissue factor expression in human endothelial cells by CD40 ligand is mediated via activator protein 1, nuclear factor kappa B, and Egr-1. *J Biol Chem, 277*(28), 25032-25039.

Bazan, J. F. (1990). Structural design and molecular evolution of a cytokine receptor superfamily. *Proc Natl Acad Sci U S A, 87*(18), 6934-6938.

Bea, F., Blessing, E., Shelley, M. I., Shultz, J. M., & Rosenfeld, M. E. (2003). Simvastatin inhibits expression of tissue factor in advanced atherosclerotic lesions of apolipoprotein E deficient mice independently of lipid lowering: potential role of simvastatin-mediated inhibition of Egr-1 expression and activation. *Atherosclerosis, 167*(2), 187-194.

Bergum, P. W., Cruikshank, A., Maki, S. L., Kelly, C. R., Ruf, W., & Vlasuk, G. P. (2001). Role of zymogen and activated factor X as scaffolds for the inhibition of the blood coagulation factor VIIa-tissue factor complex by recombinant nematode anticoagulant protein c2. *J Biol Chem, 276*(13), 10063-10071.

Bierhaus, A., Chevion, S., Chevion, M., Hofmann, M., Quehenberger, P., Illmer, T., et al. (1997). Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. *Diabetes, 46*(9), 1481-1490.

Bierhaus, A., Illmer, T., Kasper, M., Luther, T., Quehenberger, P., Tritschler, H., et al. (1997). Advanced glycation end product (AGE)-mediated induction of tissue factor in cultured endothelial cells is dependent on RAGE. *Circulation, 96*(7), 2262-2271.

Biro, E., Sturk-Maquelin, K. N., Vogel, G. M., Meuleman, D. G., Smit, M. J., Hack, C. E., et al. (2003). Human cell-derived microparticles promote thrombus formation in vivo in a tissue factor-dependent manner. *J Thromb Haemost, 1*(12), 2561-2568.

Blum, S., Issbruker, K., Willuweit, A., Hehlgans, S., Lucerna, M., Mechtcheriakova, D., et al. (2001). An inhibitory role of the phosphatidylinositol 3-kinase-signaling pathway in vascular endothelial growth factor-induced tissue factor expression. *J Biol Chem, 276*(36), 33428-33434.

Boeri, D., Almus, F. E., Maiello, M., Cagliero, E., Rao, L. V., & Lorenzi, M. (1989). Modification of tissue-factor mRNA and protein response to thrombin and interleukin 1 by high glucose in cultured human endothelial cells. *Diabetes, 38*(2), 212-218.

Bogdanov, V. Y., Balasubramanian, V., Hathcock, J., Vele, O., Lieb, M., & Nemerson, Y. (2003). Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. *Nat Med, 9*(4), 458-462.

Bogdanov, V. Y., Kirk, R. I., Miller, C., Hathcock, J. J., Vele, S., Gazdoiu, M., et al. (2006). Identification and characterization of murine alternatively spliced tissue factor. *J Thromb Haemost, 4*(1), 158-167.

Brand, K., Fowler, B. J., Edgington, T. S., & Mackman, N. (1991). Tissue factor mRNA in THP-1 monocytic cells is regulated at both transcriptional and posttranscriptional levels in response to lipopolysaccharide. *Mol Cell Biol, 11*(9), 4732-4738.

Brandes, R. P., Beer, S., Ha, T., & Busse, R. (2003). Withdrawal of cerivastatin induces monocyte chemoattractant protein 1 and tissue factor expression in

cultured vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol, 23*(10), 1794-1800.

Breitenstein, A., Camici, G. G., & Tanner, F. C. (2010). Tissue factor: beyond coagulation in the cardiovascular system. *Clin Sci (Lond), 118*(3), 159-172.

Breitenstein, A., Stampfli, S. F., Camici, G. G., Akhmedov, A., Ha, H. R., Follath, F., et al. (2008). Amiodarone inhibits arterial thrombus formation and tissue factor translation. *Arterioscler Thromb Vasc Biol, 28*(12), 2231-2238.

Breitenstein, A., Tanner, F. C., & Luscher, T. F. (2010). Tissue factor and cardiovascular disease: quo vadis? *Circ J, 74*(1), 3-12.

Bromberg, M. E., Konigsberg, W. H., Madison, J. F., Pawashe, A., & Garen, A. (1995). Tissue factor promotes melanoma metastasis by a pathway independent of blood coagulation. *Proc Natl Acad Sci U S A, 92*(18), 8205-8209.

Broze, G. J., Jr., Leykam, J. E., Schwartz, B. D., & Miletich, J. P. (1985). Purification of human brain tissue factor. *J Biol Chem, 260*(20), 10917-10920.

Brunetti, N. D., Munno, I., Pellegrino, P. L., Ruggiero, V., Correale, M., Cuculo, A., et al. (2007). Inflammatory cytokine imbalance after coronary angioplasty: links with coronary atherosclerosis. *J Interv Cardiol, 20*(4), 248-257.

Butenas, S., Bouchard, B. A., Brummel-Ziedins, K. E., Parhami-Seren, B., & Mann, K. G. (2005). Tissue factor activity in whole blood. *Blood, 105*(7), 2764-2770.

Cai, H., Song, C., Endoh, I., Goyette, J., Jessup, W., Freedman, S. B., et al. (2007). Serum amyloid A induces monocyte tissue factor. *J Immunol, 178*(3), 1852-1860.

Camera, M., Frigerio, M., Toschi, V., Brambilla, M., Rossi, F., Cottell, D. C., et al. (2003). Platelet activation induces cell-surface immunoreactive tissue factor expression, which is modulated differently by antiplatelet drugs. *Arterioscler Thromb Vasc Biol, 23*(9), 1690-1696.

Camerer, E., Gjernes, E., Wiiger, M., Pringle, S., & Prydz, H. (2000). Binding of factor VIIa to tissue factor on keratinocytes induces gene expression. *J Biol Chem, 275*(9), 6580-6585.

Camerer, E., Rottingen, J. A., Gjernes, E., Larsen, K., Skartlien, A. H., Iversen, J. G., et al. (1999). Coagulation factors VIIa and Xa induce cell signaling leading to up-regulation of the egr-1 gene. *J Biol Chem, 274*(45), 32225-32233.

Camerer, E., Rottingen, J. A., Iversen, J. G., & Prydz, H. (1996). Coagulation factors VII and X induce Ca2+ oscillations in Madin-Darby canine kidney cells only when proteolytically active. *J Biol Chem, 271*(46), 29034-29042.

Camici, G. G., Steffel, J., Akhmedov, A., Schafer, N., Baldinger, J., Schulz, U., et al. (2006). Dimethyl sulfoxide inhibits tissue factor expression, thrombus formation, and vascular smooth muscle cell activation: a potential treatment strategy for drug-eluting stents. *Circulation, 114*(14), 1512-1521.

Camici, G. G., Steffel, J., Amanovic, I., Breitenstein, A., Baldinger, J., Keller, S., et al. (2010). Rapamycin promotes arterial thrombosis in vivo: implications for everolimus and zotarolimus eluting stents. *Eur Heart J, 31*(2), 236-242.

Cavusoglu, E., Chen, I., Rappaport, J., & Marmur, J. D. (2002). Inhibition of tissue factor gene induction and activity using a hairpin ribozyme. *Circulation, 105*(19), 2282-2287.

Censarek, P., Bobbe, A., Grandoch, M., Schror, K., & Weber, A. A. (2007). Alternatively spliced human tissue factor (asHTF) is not pro-coagulant. *Thromb Haemost, 97*(1), 11-14.

Cermak, J., Key, N. S., Bach, R. R., Balla, J., Jacob, H. S., & Vercellotti, G. M. (1993). C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood, 82*(2), 513-520.

Chou, J., Mackman, N., Merrill-Skoloff, G., Pedersen, B., Furie, B. C., & Furie, B. (2004). Hematopoietic cell-derived microparticle tissue factor contributes to fibrin formation during thrombus propagation. *Blood, 104*(10), 3190-3197.

Creasey, A. A., Chang, A. C., Feigen, L., Wun, T. C., Taylor, F. B., Jr., & Hinshaw, L. B. (1993). Tissue factor pathway inhibitor reduces mortality from Escherichia coli septic shock. *J Clin Invest, 91*(6), 2850-2860.

Cugno, M., Marzano, A. V., Tedeschi, A., Fanoni, D., Venegoni, L., & Asero, R. (2009). Expression of tissue factor by eosinophils in patients with chronic urticaria. *Int Arch Allergy Immunol, 148*(2), 170-174.

Day, S. M., Reeve, J. L., Pedersen, B., Farris, D. M., Myers, D. D., Im, M., et al. (2005). Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood, 105*(1), 192-198.

Diamant, M., Nieuwland, R., Pablo, R. F., Sturk, A., Smit, J. W., & Radder, J. K. (2002). Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes mellitus. *Circulation, 106*(19), 2442-2447.

Dielis, A. W., Smid, M., Spronk, H. M., Hamulyak, K., Kroon, A. A., ten Cate, H., et al. (2005). The prothrombotic paradox of hypertension: role of the renin-angiotensin and kallikrein-kinin systems. *Hypertension, 46*(6), 1236-1242.

Drake, T. A., Morrissey, J. H., & Edgington, T. S. (1989). Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol, 134*(5), 1087-1097.

Durrington, P. (2003). Dyslipidaemia. *Lancet, 362*(9385), 717-731.

Edgington, T. S., Ruf, W., Rehemtulla, A., & Mackman, N. (1991). The molecular biology of initiation of coagulation by tissue factor. *Curr Stud Hematol Blood Transfus*(58), 15-21.

El-Ghoroury, E. A., El-Din, H. G., Abdel-Kader, M., & Ragab, S. (2008). Study of factor VII, tissue factor pathway inhibitor and monocyte tissue factor in noninsulin-dependent diabetes mellitus. *Blood Coagul Fibrinolysis, 19*(1), 7-13.

Erhardtsen, E., Nilsson, P., Johannessen, M., & Thomsen, M. S. (2001). Pharmacokinetics and safety of FFR-rFVIIa after single doses in healthy subjects. *J Clin Pharmacol, 41*(8), 880-885.

Erlich, J. H., Boyle, E. M., Labriola, J., Kovacich, J. C., Santucci, R. A., Fearns, C., et al. (2000). Inhibition of the tissue factor-thrombin pathway limits infarct size after myocardial ischemia-reperfusion injury by reducing inflammation. *Am J Pathol, 157*(6), 1849-1862.

Eto, M., Kouroedov, A., Cosentino, F., & Luscher, T. F. (2005). Glycogen synthase kinase-3 mediates endothelial cell activation by tumor necrosis factor-alpha. *Circulation, 112*(9), 1316-1322.

Eto, M., Kozai, T., Cosentino, F., Joch, H., & Luscher, T. F. (2002). Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. *Circulation, 105*(15), 1756-1759.

Falati, S., Liu, Q., Gross, P., Merrill-Skoloff, G., Chou, J., Vandendries, E., et al. (2003). Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J Exp Med, 197*(11), 1585-1598.

Fei, H., Berliner, J. A., Parhami, F., & Drake, T. A. (1993). Regulation of endothelial cell tissue factor expression by minimally oxidized LDL and lipopolysaccharide. *Arterioscler Thromb, 13*(11), 1711-1717.

Felmeden, D. C., Spencer, C. G., Chung, N. A., Belgore, F. M., Blann, A. D., Beevers, D. G., et al. (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*(4), 400-405.

Ferrara, N., & Davis-Smyth, T. (1997). The biology of vascular endothelial growth factor. *Endocr Rev, 18*(1), 4-25.

Fleck, R. A., Rao, L. V., Rapaport, S. I., & Varki, N. (1990). Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. *Thromb Res, 59*(2), 421-437.

Fluture, A., Giugliano, G. R., & Giugliano, R. P. (2007). Recombinant nematode anticoagulant protein c2 in non-ST segment elevation acute coronary syndrome and beyond. *Future Cardiol, 3*(4), 365-375.

Fu, Y., Zhang, Z., Zhang, G., Liu, Y., Cao, Y., Yu, J., et al. (2008). Adenovirus-mediated gene transfer of tissue factor pathway inhibitor induces apoptosis in vascular smooth muscle cells. *Apoptosis, 13*(5), 634-640.

Gailani, D., & Renne, T. (2007). Intrinsic pathway of coagulation and arterial thrombosis. *Arterioscler Thromb Vasc Biol, 27*(12), 2507-2513.

Gebhard, C., Stampfli, S. F., Gebhard, C. E., Akhmedov, A., Breitenstein, A., Camici, G. G., et al. (2009). Guggulsterone, an anti-inflammatory phytosterol, inhibits tissue factor and arterial thrombosis. *Basic Res Cardiol, 104*(3), 285-294.

Giugliano, R. P., Wiviott, S. D., Stone, P. H., Simon, D. I., Schweiger, M. J., Bouchard, A., et al. (2007). Recombinant nematode anticoagulant protein c2 in patients with non-ST-segment elevation acute coronary syndrome: the ANTHEM-TIMI-32 trial. *J Am Coll Cardiol, 49*(25), 2398-2407.

Golino, P., Ragni, M., Cirillo, P., D'Andrea, D., Scognamiglio, A., Ravera, A., et al. (1998). Antithrombotic effects of recombinant human, active site-blocked factor VIIa in a rabbit model of recurrent arterial thrombosis. *Circ Res, 82*(1), 39-46.

Golledge, J., Mangan, S., & Clancy, P. (2007). Effects of peroxisome proliferator-activated receptor ligands in modulating tissue factor and tissue factor pathway inhibitor in acutely symptomatic carotid atheromas. *Stroke, 38*(5), 1501-1508.

Guba, M., Yezhelyev, M., Eichhorn, M. E., Schmid, G., Ischenko, I., Papyan, A., et al. (2005). Rapamycin induces tumor-specific thrombosis via tissue factor in the presence of VEGF. *Blood, 105*(11), 4463-4469.

Guha, M., & Mackman, N. (2002). The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of

inflammatory mediators in human monocytic cells. *J Biol Chem, 277*(35), 32124-32132.

Hamuro, T., Kamikubo, Y., Nakahara, Y., Miyamoto, S., & Funatsu, A. (1998). Human recombinant tissue factor pathway inhibitor induces apoptosis in cultured human endothelial cells. *FEBS Lett, 421*(3), 197-202.

Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med, 352*(16), 1685-1695.

Harker, L. A., Hanson, S. R., Wilcox, J. N., & Kelly, A. B. (1996). Antithrombotic and antilesion benefits without hemorrhagic risks by inhibiting tissue factor pathway. *Haemostasis, 26 Suppl 1*, 76-82.

Hartzell, S., Ryder, K., Lanahan, A., Lau, L. F., & Nathan, D. (1989). A growth factor-responsive gene of murine BALB/c 3T3 cells encodes a protein homologous to human tissue factor. *Mol Cell Biol, 9*(6), 2567-2573.

Hathcock, J. J., & Nemerson, Y. (2004). Platelet deposition inhibits tissue factor activity: in vitro clots are impermeable to factor Xa. *Blood, 104*(1), 123-127.

He, M., He, X., Xie, Q., Chen, F., & He, S. (2006). Angiotensin II induces the expression of tissue factor and its mechanism in human monocytes. *Thromb Res, 117*(5), 579-590.

He, Y., Chang, G., Zhan, S., Song, X., Wang, X., & Luo, Y. (2008). Soluble tissue factor has unique angiogenic activities that selectively promote migration and differentiation but not proliferation of endothelial cells. *Biochem Biophys Res Commun, 370*(3), 489-494.

Himber, J., Wohlgensinger, C., Roux, S., Damico, L. A., Fallon, J. T., Kirchhofer, D., et al. (2003). Inhibition of tissue factor limits the growth of venous thrombus in the rabbit. *J Thromb Haemost, 1*(5), 889-895.

Hoffman, M., Whinna, H. C., & Monroe, D. M. (2006). Circulating tissue factor accumulates in thrombi, but not in hemostatic plugs. *J Thromb Haemost, 4*(9), 2092-2093.

Holy, E. W., Akhmedov, A., Luscher, T. F., & Tanner, F. C. (2009). Berberine, a natural lipid-lowering drug, exerts prothrombotic effects on vascular cells. *J Mol Cell Cardiol, 46*(2), 234-240.

Holy, E. W., Stampfli, S. F., Akhmedov, A., Holm, N., Camici, G. G., Luscher, T. F., et al. (2009). Laminin receptor activation inhibits endothelial tissue factor expression. *J Mol Cell Cardiol*.

Hron, G., Kollars, M., Weber, H., Sagaster, V., Quehenberger, P., Eichinger, S., et al. (2007). Tissue factor-positive microparticles: cellular origin and association with coagulation activation in patients with colorectal cancer. *Thromb Haemost, 97*(1), 119-123.

Iakovou, I., Schmidt, T., Bonizzoni, E., Ge, L., Sangiorgi, G. M., Stankovic, G., et al. (2005). Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA, 293*(17), 2126-2130.

Jang, Y., Guzman, L. A., Lincoff, A. M., Gottsauner-Wolf, M., Forudi, F., Hart, C. E., et al. (1995). Influence of blockade at specific levels of the coagulation cascade on restenosis in a rabbit atherosclerotic femoral artery injury model. *Circulation, 92*(10), 3041-3050.

Jedsadayanmata, A., Chen, C. C., Kireeva, M. L., Lau, L. F., & Lam, S. C. (1999). Activation-dependent adhesion of human platelets to Cyr61 and Fisp12/mouse connective tissue growth factor is mediated through integrin alpha(IIb)beta(3). *J Biol Chem, 274*(34), 24321-24327.

Jesty, J., & Beltrami, E. (2005). Positive feedbacks of coagulation: their role in threshold regulation. *Arterioscler Thromb Vasc Biol, 25*(12), 2463-2469.

Jiao, J. A., Kelly, A. B., Marzec, U. M., Nieves, E., Acevedo, J., Burkhardt, M., et al. (2010). Inhibition of acute vascular thrombosis in chimpanzees by an anti-human tissue factor antibody targeting the factor X binding site. *Thromb Haemost, 103*(1), 224-233.

Kambas, K., Markiewski, M. M., Pneumatikos, I. A., Rafail, S. S., Theodorou, V., Konstantonis, D., et al. (2008). C5a and TNF-alpha up-regulate the expression of tissue factor in intra-alveolar neutrophils of patients with the acute respiratory distress syndrome. *J Immunol, 180*(11), 7368-7375.

Kamimura, M., Bea, F., Akizawa, T., Katus, H. A., Kreuzer, J., & Viedt, C. (2004). Platelet-derived growth factor induces tissue factor expression in vascular smooth muscle cells via activation of Egr-1. *Hypertension, 44*(6), 944-951.

Kao, F. T., Hartz, J., Horton, R., Nemerson, Y., & Carson, S. D. (1988). Regional assignment of human tissue factor gene (F3) to chromosome 1p21-p22. *Somat Cell Mol Genet, 14*(4), 407-410.

Kislinger, T., Tanji, N., Wendt, T., Qu, W., Lu, Y., Ferran, L. J., Jr., et al. (2001). Receptor for advanced glycation end products mediates inflammation and

enhanced expression of tissue factor in vasculature of diabetic apolipoprotein E-null mice. *Arterioscler Thromb Vasc Biol, 21*(6), 905-910.

Koh, K. K., Chung, W. J., Ahn, J. Y., Han, S. H., Kang, W. C., Seo, Y. H., et al. (2004). Angiotensin II type 1 receptor blockers reduce tissue factor activity and plasminogen activator inhibitor type-1 antigen in hypertensive patients: a randomized, double-blind, placebo-controlled study. *Atherosclerosis, 177*(1), 155-160.

Lechner, D., Kollars, M., Gleiss, A., Kyrle, P. A., & Weltermann, A. (2007). Chemotherapy-induced thrombin generation via procoagulant endothelial microparticles is independent of tissue factor activity. *J Thromb Haemost, 5*(12), 2445-2452.

Lee, K. W., Blann, A. D., & Lip, G. Y. (2005). Plasma markers of endothelial damage/dysfunction, inflammation and thrombogenesis in relation to TIMI risk stratification in acute coronary syndromes. *Thromb Haemost, 94*(5), 1077-1083.

Lev, E. I., Marmur, J. D., Zdravkovic, M., Osende, J. I., Robbins, J., Delfin, J. A., et al. (2002). Antithrombotic effect of tissue factor inhibition by inactivated factor VIIa: an ex vivo human study. *Arterioscler Thromb Vasc Biol, 22*(6), 1036-1041.

Lewis, J. C., Bennett-Cain, A. L., DeMars, C. S., Doellgast, G. J., Grant, K. W., Jones, N. L., et al. (1995). Procoagulant activity after exposure of monocyte-derived macrophages to minimally oxidized low density lipoprotein. Co-localization of tissue factor antigen and nascent fibrin fibers at the cell surface. *Am J Pathol, 147*(4), 1029-1040.

Lim, H. S., Blann, A. D., & Lip, G. Y. (2004). Soluble CD40 ligand, soluble P-selectin, interleukin-6, and tissue factor in diabetes mellitus: relationships to cardiovascular disease and risk factor intervention. *Circulation, 109*(21), 2524-2528.

Llorente-Cortes, V., Otero-Vinas, M., Camino-Lopez, S., Llampayas, O., & Badimon, L. (2004). Aggregated low-density lipoprotein uptake induces membrane tissue factor procoagulant activity and microparticle release in human vascular smooth muscle cells. *Circulation, 110*(4), 452-459.

Luyendyk, J. P., Schabbauer, G. A., Tencati, M., Holscher, T., Pawlinski, R., & Mackman, N. (2008). Genetic analysis of the role of the PI3K-Akt pathway in lipopolysaccharide-induced cytokine and tissue factor gene expression in monocytes/macrophages. *J Immunol, 180*(6), 4218-4226.

Mach, F., Schonbeck, U., Bonnefoy, J. Y., Pober, J. S., & Libby, P. (1997). Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor. *Circulation, 96*(2), 396-399.

Mackman, N. (1997). Regulation of the tissue factor gene. *Thromb Haemost, 78*(1), 747-754.

Mackman, N. (2007). Alternatively spliced tissue factor - one cut too many? *Thromb Haemost, 97*(1), 5-8.

Mackman, N. (2009). The role of tissue factor and factor VIIa in hemostasis. *Anesth Analg, 108*(5), 1447-1452.

Mackman, N., Morrissey, J. H., Fowler, B., & Edgington, T. S. (1989). Complete sequence of the human tissue factor gene, a highly regulated cellular receptor that initiates the coagulation protease cascade. *Biochemistry, 28*(4), 1755-1762.

Mackman, N., Sawdey, M. S., Keeton, M. R., & Loskutoff, D. J. (1993). Murine tissue factor gene expression in vivo. Tissue and cell specificity and regulation by lipopolysaccharide. *Am J Pathol, 143*(1), 76-84.

Mackman, N., Tilley, R. E., & Key, N. S. (2007). Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol, 27*(8), 1687-1693.

Mallat, Z., Benamer, H., Hugel, B., Benessiano, J., Steg, P. G., Freyssinet, J. M., et al. (2000). Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation, 101*(8), 841-843.

Maragoudakis, M. E., Tsopanoglou, N. E., & Andriopoulou, P. (2002). Mechanism of thrombin-induced angiogenesis. *Biochem Soc Trans, 30*(2), 173-177.

Markle, R. A., Han, J., Summers, B. D., Yokoyama, T., Hajjar, K. A., Hajjar, D. P., et al. (2003). Pitavastatin alters the expression of thrombotic and fibrinolytic proteins in human vascular cells. *J Cell Biochem, 90*(1), 23-32.

Marmur, J. D., Thiruvikraman, S. V., Fyfe, B. S., Guha, A., Sharma, S. K., Ambrose, J. A., et al. (1996). Identification of active tissue factor in human coronary atheroma. *Circulation, 94*(6), 1226-1232.

Marutsuka, K., Hatakeyama, K., Sato, Y., Yamashita, A., Sumiyoshi, A., & Asada, Y. (2002). Protease-activated receptor 2 (PAR2) mediates vascular smooth muscle

cell migration induced by tissue factor/factor VIIa complex. *Thromb Res, 107*(5), 271-276.

Marx, N., Mackman, N., Schonbeck, U., Yilmaz, N., Hombach, V., Libby, P., et al. (2001). PPARalpha activators inhibit tissue factor expression and activity in human monocytes. *Circulation, 103*(2), 213-219.

Maugeri, N., Brambilla, M., Camera, M., Carbone, A., Tremoli, E., Donati, M. B., et al. (2006). Human polymorphonuclear leukocytes produce and express functional tissue factor upon stimulation. *J Thromb Haemost, 4*(6), 1323-1330.

Maugeri, N., Giordano, G., Petrilli, M. P., Fraticelli, V., de Gaetano, G., Cerletti, C., et al. (2006). Inhibition of tissue factor expression by hydroxyurea in polymorphonuclear leukocytes from patients with myeloproliferative disorders: a new effect for an old drug? *J Thromb Haemost, 4*(12), 2593-2598.

Mechtcheriakova, D., Schabbauer, G., Lucerna, M., Clauss, M., De Martin, R., Binder, B. R., et al. (2001). Specificity, diversity, and convergence in VEGF and TNF-alpha signaling events leading to tissue factor up-regulation via EGR-1 in endothelial cells. *FASEB J, 15*(1), 230-242.

Miller, D. L., Welty-Wolf, K., Carraway, M. S., Ezban, M., Ghio, A., Suliman, H., et al. (2002). Extrinsic coagulation blockade attenuates lung injury and proinflammatory cytokine release after intratracheal lipopolysaccharide. *Am J Respir Cell Mol Biol, 26*(6), 650-658.

Misumi, K., Ogawa, H., Yasue, H., Soejima, H., Suefuji, H., Nishiyama, K., et al. (1998). Comparison of plasma tissue factor levels in unstable and stable angina pectoris. *Am J Cardiol, 81*(1), 22-26.

Monetti, M., Canavesi, M., Camera, M., Parente, R., Paoletti, R., Tremoli, E., et al. (2007). Rosuvastatin displays anti-atherothrombotic and anti-inflammatory properties in apoE-deficient mice. *Pharmacol Res, 55*(5), 441-449.

Moons, A. H., Peters, R. J., Bijsterveld, N. R., Piek, J. J., Prins, M. H., Vlasuk, G. P., et al. (2003). Recombinant nematode anticoagulant protein c2, an inhibitor of the tissue factor/factor VIIa complex, in patients undergoing elective coronary angioplasty. *J Am Coll Cardiol, 41*(12), 2147-2153.

Moosbauer, C., Morgenstern, E., Cuvelier, S. L., Manukyan, D., Bidzhekov, K., Albrecht, S., et al. (2007). Eosinophils are a major intravascular location for tissue factor storage and exposure. *Blood, 109*(3), 995-1002.

Morrissey, J. H., Gregory, S. A., Mackman, N., & Edgington, T. S. (1989). Tissue factor regulation and gene organization. *Oxf Surv Eukaryot Genes, 6*, 67-84.

Morrow, D. A., Murphy, S. A., McCabe, C. H., Mackman, N., Wong, H. C., & Antman, E. M. (2005). Potent inhibition of thrombin with a monoclonal antibody against tissue factor (Sunol-cH36): results of the PROXIMATE-TIMI 27 trial. *Eur Heart J, 26*(7), 682-688.

Mueller, B. M., & Ruf, W. (1998). Requirement for binding of catalytically active factor VIIa in tissue factor-dependent experimental metastasis. *J Clin Invest, 101*(7), 1372-1378.

Muller, I., Klocke, A., Alex, M., Kotzsch, M., Luther, T., Morgenstern, E., et al. (2003). Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. *FASEB J, 17*(3), 476-478.

Napoleone, E., Di Santo, A., Camera, M., Tremoli, E., & Lorenzet, R. (2000). Angiotensin-converting enzyme inhibitors downregulate tissue factor synthesis in monocytes. *Circ Res, 86*(2), 139-143.

Napoleone, E., Di Santo, A., & Lorenzet, R. (1997). Monocytes upregulate endothelial cell expression of tissue factor: a role for cell-cell contact and cross-talk. *Blood, 89*(2), 541-549.

Ngo, C. V., Picha, K., McCabe, F., Millar, H., Tawadros, R., Tam, S. H., et al. (2007). CNTO 859, a humanized anti-tissue factor monoclonal antibody, is a potent inhibitor of breast cancer metastasis and tumor growth in xenograft models. *Int J Cancer, 120*(6), 1261-1267.

Nishida, T., Ueno, H., Atsuchi, N., Kawano, R., Asada, Y., Nakahara, Y., et al. (1999). Adenovirus-mediated local expression of human tissue factor pathway inhibitor eliminates shear stress-induced recurrent thrombosis in the injured carotid artery of the rabbit. *Circ Res, 84*(12), 1446-1452.

Oemar, B. S., & Luscher, T. F. (1997). Connective tissue growth factor. Friend or foe? *Arterioscler Thromb Vasc Biol, 17*(8), 1483-1489.

Oemar, B. S., Werner, A., Garnier, J. M., Do, D. D., Godoy, N., Nauck, M., et al. (1997). Human connective tissue growth factor is expressed in advanced atherosclerotic lesions. *Circulation, 95*(4), 831-839.

Okorie, U. M., Denney, W. S., Chatterjee, M. S., Neeves, K. B., & Diamond, S. L. (2008). Determination of surface tissue factor thresholds that trigger coagulation at venous and arterial shear rates: amplification of 100 fM circulating tissue factor requires flow. *Blood, 111*(7), 3507-3513.

Orfeo, T., Butenas, S., Brummel-Ziedins, K. E., & Mann, K. G. (2005). The tissue factor requirement in blood coagulation. *J Biol Chem, 280*(52), 42887-42896.

Orvim, U., Barstad, R. M., Orning, L., Petersen, L. B., Ezban, M., Hedner, U., et al. (1997). Antithrombotic efficacy of inactivated active site recombinant factor VIIa is shear dependent in human blood. *Arterioscler Thromb Vasc Biol, 17*(11), 3049-3056.

Osterud, B., & Bjorklid, E. (2001). The tissue factor pathway in disseminated intravascular coagulation. *Semin Thromb Hemost, 27*(6), 605-617.

Ott, I., Fischer, E. G., Miyagi, Y., Mueller, B. M., & Ruf, W. (1998). A role for tissue factor in cell adhesion and migration mediated by interaction with actin-binding protein 280. *J Cell Biol, 140*(5), 1241-1253.

Ott, I., Michaelis, C., Schuermann, M., Steppich, B., Seitz, I., Dewerchin, M., et al. (2005). Vascular remodeling in mice lacking the cytoplasmic domain of tissue factor. *Circ Res, 97*(3), 293-298.

Paborsky, L. R., Caras, I. W., Fisher, K. L., & Gorman, C. M. (1991). Lipid association, but not the transmembrane domain, is required for tissue factor activity. Substitution of the transmembrane domain with a phosphatidylinositol anchor. *J Biol Chem, 266*(32), 21911-21916.

Panes, O., Matus, V., Saez, C. G., Quiroga, T., Pereira, J., & Mezzano, D. (2007). Human platelets synthesize and express functional tissue factor. *Blood, 109*(12), 5242-5250.

Pawlinski, R., Fernandes, A., Kehrle, B., Pedersen, B., Parry, G., Erlich, J., et al. (2002). Tissue factor deficiency causes cardiac fibrosis and left ventricular dysfunction. *Proc Natl Acad Sci U S A, 99*(24), 15333-15338.

Pawlinski, R., Pedersen, B., Schabbauer, G., Tencati, M., Holscher, T., Boisvert, W., et al. (2004). Role of tissue factor and protease-activated receptors in a mouse model of endotoxemia. *Blood, 103*(4), 1342-1347.

Pedersen, B., Holscher, T., Sato, Y., Pawlinski, R., & Mackman, N. (2005). A balance between tissue factor and tissue factor pathway inhibitor is required for embryonic development and hemostasis in adult mice. *Blood, 105*(7), 2777-2782.

Pendurthi, U. R., Allen, K. E., Ezban, M., & Rao, L. V. (2000). Factor VIIa and thrombin induce the expression of Cyr61 and connective tissue growth factor, extracellular matrix signaling proteins that could act as possible downstream mediators in factor VIIa x tissue factor-induced signal transduction. *J Biol Chem, 275*(19), 14632-14641.

Pendurthi, U. R., Alok, D., & Rao, L. V. (1997). Binding of factor VIIa to tissue factor induces alterations in gene expression in human fibroblast cells: up-regulation of poly(A) polymerase. *Proc Natl Acad Sci U S A, 94*(23), 12598-12603.

Penn, M. S., Patel, C. V., Cui, M. Z., DiCorleto, P. E., & Chisolm, G. M. (1999). LDL increases inactive tissue factor on vascular smooth muscle cell surfaces: hydrogen peroxide activates latent cell surface tissue factor. *Circulation, 99*(13), 1753-1759.

Perez-Pujol, S., Aras, O., Lozano, M., Cocking-Johnson, D., Key, N. S., White, J. G., et al. (2005). Stored platelets contain residual amounts of tissue factor: evidence

from studies on platelet concentrates stored for prolonged periods. *Transfusion, 45*(4), 572-579.

Poulsen, L. K., Jacobsen, N., Sorensen, B. B., Bergenhem, N. C., Kelly, J. D., Foster, D. C., et al. (1998). Signal transduction via the mitogen-activated protein kinase pathway induced by binding of coagulation factor VIIa to tissue factor. *J Biol Chem, 273*(11), 6228-6232.

Ragni, M., Cirillo, P., Pascucci, I., Scognamiglio, A., D'Andrea, D., Eramo, N., et al. (1996). Monoclonal antibody against tissue factor shortens tissue plasminogen activator lysis time and prevents reocclusion in a rabbit model of carotid artery thrombosis. *Circulation, 93*(10), 1913-1918.

Ragni, M., Golino, P., Cirillo, P., Pascucci, I., Scognamiglio, A., Ravera, A., et al. (1996). [Inactivated factor VII exercises a powerful antithrombotic activity in an experimental model of recurrent arterial thrombosis]. *Cardiologia, 41*(1), 51-58.

Rauch, U., Bonderman, D., Bohrmann, B., Badimon, J. J., Himber, J., Riederer, M. A., et al. (2000). Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. *Blood, 96*(1), 170-175.

Rehemtulla, A., Ruf, W., & Edgington, T. S. (1991). The integrity of the cysteine 186-cysteine 209 bond of the second disulfide loop of tissue factor is required for binding of factor VII. *J Biol Chem, 266*(16), 10294-10299.

Riewald, M., & Ruf, W. (2002). Orchestration of coagulation protease signaling by tissue factor. *Trends Cardiovasc Med, 12*(4), 149-154.

Rottingen, J. A., Enden, T., Camerer, E., Iversen, J. G., & Prydz, H. (1995). Binding of human factor VIIa to tissue factor induces cytosolic Ca2+ signals in J82 cells,

transfected COS-1 cells, Madin-Darby canine kidney cells and in human endothelial cells induced to synthesize tissue factor. *J Biol Chem, 270*(9), 4650-4660.

Sambola, A., Osende, J., Hathcock, J., Degen, M., Nemerson, Y., Fuster, V., et al. (2003). Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation, 107*(7), 973-977.

Sato, Y., Asada, Y., Marutsuka, K., Hatakeyama, K., & Sumiyoshi, A. (1996). Tissue factor induces migration of cultured aortic smooth muscle cells. *Thromb Haemost, 75*(3), 389-392.

Sato, Y., Kataoka, H., Asada, Y., Marutsuka, K., Kamikubo, Y., Koono, M., et al. (1999). Overexpression of tissue factor pathway inhibitor in aortic smooth muscle cells inhibits cell migration induced by tissue factor/factor VIIa complex. *Thromb Res, 94*(6), 401-406.

Scarpati, E. M., Wen, D., Broze, G. J., Jr., Miletich, J. P., Flandermeyer, R. R., Siegel, N. R., et al. (1987). Human tissue factor: cDNA sequence and chromosome localization of the gene. *Biochemistry, 26*(17), 5234-5238.

Schecter, A. D., Giesen, P. L., Taby, O., Rosenfield, C. L., Rossikhina, M., Fyfe, B. S., et al. (1997). Tissue factor expression in human arterial smooth muscle cells. TF is present in three cellular pools after growth factor stimulation. *J Clin Invest, 100*(9), 2276-2285.

Schecter, A. D., Spirn, B., Rossikhina, M., Giesen, P. L., Bogdanov, V., Fallon, J. T., et al. (2000). Release of active tissue factor by human arterial smooth muscle cells. *Circ Res, 87*(2), 126-132.

Schober, J. M., Chen, N., Grzeszkiewicz, T. M., Jovanovic, I., Emeson, E. E., Ugarova, T. P., et al. (2002). Identification of integrin alpha(M)beta(2) as an adhesion receptor on peripheral blood monocytes for Cyr61 (CCN1) and connective tissue growth factor (CCN2): immediate-early gene products expressed in atherosclerotic lesions. *Blood, 99*(12), 4457-4465.

Siddiqui, F. A., Desai, H., Amirkhosravi, A., Amaya, M., & Francis, J. L. (2002). The presence and release of tissue factor from human platelets. *Platelets, 13*(4), 247-253.

Siegbahn, A. (2000). Cellular consequences upon factor VIIa binding to tissue factor. *Haemostasis, 30 Suppl 2*, 41-47.

Singh, R., Pan, S., Mueske, C. S., Witt, T., Kleppe, L. S., Peterson, T. E., et al. (2001). Role for tissue factor pathway in murine model of vascular remodeling. *Circ Res, 89*(1), 71-76.

Soejima, H., Ogawa, H., Yasue, H., Kaikita, K., Nishiyama, K., Misumi, K., et al. (1999). Heightened tissue factor associated with tissue factor pathway inhibitor and prognosis in patients with unstable angina. *Circulation, 99*(22), 2908-2913.

Soejima, H., Ogawa, H., Yasue, H., Suefuji, H., Kaikita, K., Tsuji, I., et al. (1996). Effects of enalapril on tissue factor in patients with uncomplicated acute myocardial infarction. *Am J Cardiol, 78*(3), 336-340.

Sorensen, B. B., Persson, E., Freskgard, P. O., Kjalke, M., Ezban, M., Williams, T., et al. (1997). Incorporation of an active site inhibitor in factor VIIa alters the affinity for tissue factor. *J Biol Chem, 272*(18), 11863-11868.

St Pierre, J., Yang, L. Y., Tamirisa, K., Scherrer, D., De Ciechi, P., Eisenberg, P., et al. (1999). Tissue factor pathway inhibitor attenuates procoagulant activity and upregulation of tissue factor at the site of balloon-induced arterial injury in pigs. *Arterioscler Thromb Vasc Biol, 19*(9), 2263-2268.

Stahli, B. E., Breitenstein, A., Akhmedov, A., Camici, G. G., Shojaati, K., Bogdanov, N., et al. (2007). Cardiac glycosides regulate endothelial tissue factor expression in culture. *Arterioscler Thromb Vasc Biol, 27*(12), 2769-2776.

Stahli, B. E., Camici, G. G., Steffel, J., Akhmedov, A., Shojaati, K., Graber, M., et al. (2006). Paclitaxel enhances thrombin-induced endothelial tissue factor expression via c-Jun terminal NH2 kinase activation. *Circ Res, 99*(2), 149-155.

Steffel, J., Arnet, C., Akhmedov, A., Iseli, S. M., Luscher, T. F., & Tanner, F. C. (2006). Histamine differentially interacts with tumor necrosis factor-alpha and thrombin in endothelial tissue factor induction: the role of c-Jun NH2-terminal kinase. *J Thromb Haemost, 4*(11), 2452-2460.

Steffel, J., Hermann, M., Greutert, H., Gay, S., Luscher, T. F., Ruschitzka, F., et al. (2005). Celecoxib decreases endothelial tissue factor expression through inhibition of c-Jun terminal NH2 kinase phosphorylation. *Circulation, 111*(13), 1685-1689.

Steffel, J., Latini, R. A., Akhmedov, A., Zimmermann, D., Zimmerling, P., Luscher, T. F., et al. (2005). Rapamycin, but not FK-506, increases endothelial tissue factor expression: implications for drug-eluting stent design. *Circulation, 112*(13), 2002-2011.

Steffel, J., Luscher, T. F., & Tanner, F. C. (2006). Tissue factor in cardiovascular diseases: molecular mechanisms and clinical implications. *Circulation, 113*(5), 722-731.

Stegenga, M. E., van der Crabben, S. N., Levi, M., de Vos, A. F., Tanck, M. W., Sauerwein, H. P., et al. (2006). Hyperglycemia stimulates coagulation, whereas hyperinsulinemia impairs fibrinolysis in healthy humans. *Diabetes, 55*(6), 1807-1812.

Stephens, A. C., & Rivers, R. P. (1997). Suppression of human monocyte tissue factor synthesis by antisense oligodeoxynucleotide. *Thromb Res, 85*(5), 387-398.

Sturk-Maquelin, K. N., Nieuwland, R., Romijn, F. P., Eijsman, L., Hack, C. E., & Sturk, A. (2003). Pro- and non-coagulant forms of non-cell-bound tissue factor in vivo. *J Thromb Haemost, 1*(9), 1920-1926.

Szotowski, B., Antoniak, S., Poller, W., Schultheiss, H. P., & Rauch, U. (2005). Procoagulant soluble tissue factor is released from endothelial cells in response to inflammatory cytokines. *Circ Res, 96*(12), 1233-1239.

Taubman, M. B., Marmur, J. D., Rosenfield, C. L., Guha, A., Nichtberger, S., & Nemerson, Y. (1993). Agonist-mediated tissue factor expression in cultured vascular smooth muscle cells. Role of Ca2+ mobilization and protein kinase C activation. *J Clin Invest, 91*(2), 547-552.

Taylor, F. B., Jr., Chang, A., Ruf, W., Morrissey, J. H., Hinshaw, L., Catlett, R., et al. (1991). Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. *Circ Shock, 33*(3), 127-134.

ten Cate, H. (2000). Pathophysiology of disseminated intravascular coagulation in sepsis. *Crit Care Med, 28*(9 Suppl), S9-11.

Thiruvikraman, S. V., Guha, A., Roboz, J., Taubman, M. B., Nemerson, Y., & Fallon, J. T. (1996). In situ localization of tissue factor in human atherosclerotic plaques by binding of digoxigenin-labeled factors VIIa and X. *Lab Invest, 75*(4), 451-461.

Tsopanoglou, N. E., Andriopoulou, P., & Maragoudakis, M. E. (2002). On the mechanism of thrombin-induced angiogenesis: involvement of alphavbeta3-integrin. *Am J Physiol Cell Physiol, 283*(5), C1501-1510.

Tsopanoglou, N. E., Papaconstantinou, M. E., Flordellis, C. S., & Maragoudakis, M. E. (2004). On the mode of action of thrombin-induced angiogenesis: thrombin peptide, TP508, mediates effects in endothelial cells via alphavbeta3 integrin. *Thromb Haemost, 92*(4), 846-857.

Tutar, E., Ozcan, M., Kilickap, M., Gulec, S., Aras, O., Pamir, G., et al. (2003). Elevated whole-blood tissue factor procoagulant activity as a marker of restenosis after percutaneous transluminal coronary angioplasty and stent implantation. *Circulation, 108*(13), 1581-1584.

Vaidyula, V. R., Rao, A. K., Mozzoli, M., Homko, C., Cheung, P., & Boden, G. (2006). Effects of hyperglycemia and hyperinsulinemia on circulating tissue factor procoagulant activity and platelet CD40 ligand. *Diabetes, 55*(1), 202-208.

Versteeg, H. H., Hoedemaeker, I., Diks, S. H., Stam, J. C., Spaargaren, M., van Bergen En Henegouwen, P. M., et al. (2000). Factor VIIa/tissue factor-induced signaling via activation of Src-like kinases, phosphatidylinositol 3-kinase, and Rac. *J Biol Chem, 275*(37), 28750-28756.

Viswambharan, H., Ming, X. F., Zhu, S., Hubsch, A., Lerch, P., Vergeres, G., et al. (2004). Reconstituted high-density lipoprotein inhibits thrombin-induced endothelial tissue factor expression through inhibition of RhoA and stimulation of phosphatidylinositol 3-kinase but not Akt/endothelial nitric oxide synthase. *Circ Res, 94*(7), 918-925.

Wada, H., Kaneko, T., Wakita, Y., Minamikawa, K., Nagaya, S., Tamaki, S., et al. (1994). Effect of lipoproteins on tissue factor activity and PAI-II antigen in human monocytes and macrophages. *Int J Cardiol, 47*(1 Suppl), S21-25.

Wilcox, J. N., Smith, K. M., Schwartz, S. M., & Gordon, D. (1989). Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci U S A, 86*(8), 2839-2843.

Wu, J., Stevenson, M. J., Brown, J. M., Grunz, E. A., Strawn, T. L., & Fay, W. P. (2008). C-reactive protein enhances tissue factor expression by vascular smooth muscle cells: mechanisms and in vivo significance. *Arterioscler Thromb Vasc Biol, 28*(4), 698-704.

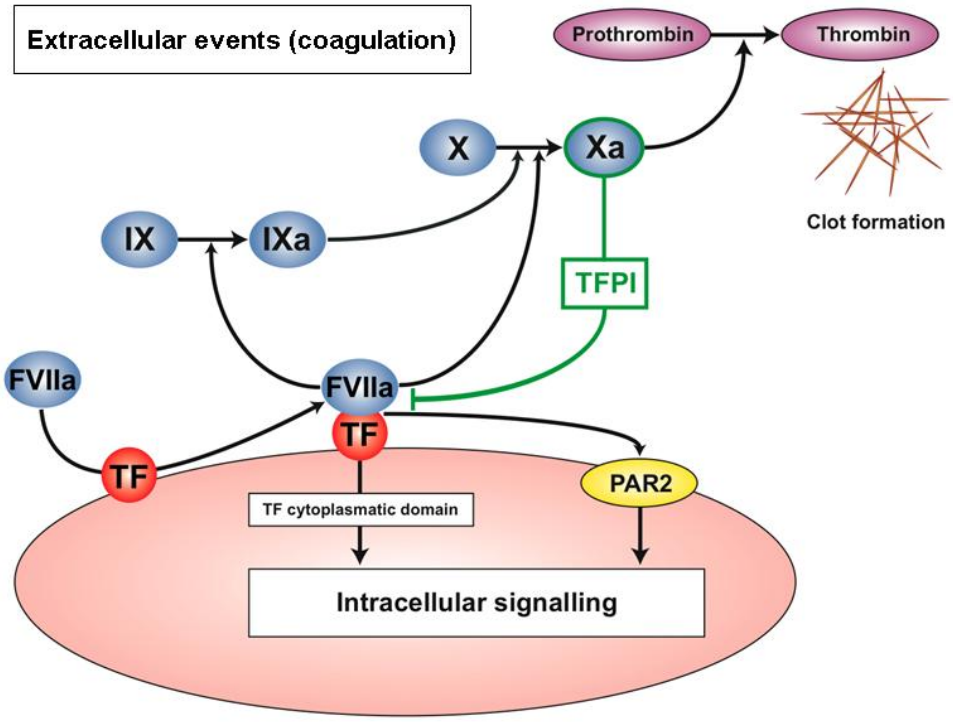
Wysokinski, W. E., Owen, W. G., Fass, D. N., Patrzalek, D. D., Murphy, L., & McBane, R. D., 2nd. (2004). Atrial fibrillation and thrombosis: immunohistochemical differences between in situ and embolized thrombi. *J Thromb Haemost, 2*(9), 1637-1644.

Zhang, J. J., Kelm, R. J., Biswas, P., Kashgarian, M., & Madri, J. A. (2007). PECAM-1 modulates thrombin-induced tissue factor expression on endothelial cells. *J Cell Physiol, 210*(2), 527-537.

Zillmann, A., Luther, T., Muller, I., Kotzsch, M., Spannagl, M., Kauke, T., et al. (2001). Platelet-associated tissue factor contributes to the collagen-triggered activation of blood coagulation. *Biochem Biophys Res Commun, 281*(2), 603-609.

Zioncheck, T. F., Roy, S., & Vehar, G. A. (1992). The cytoplasmic domain of tissue factor is phosphorylated by a protein kinase C-dependent mechanism. *J Biol Chem, 267*(6), 3561-3564.

Zoldhelyi, P., Chen, Z. Q., Shelat, H. S., McNatt, J. M., & Willerson, J. T. (2001). Local gene transfer of tissue factor pathway inhibitor regulates intimal hyperplasia in atherosclerotic arteries. *Proc Natl Acad Sci U S A, 98*(7), 4078-4083.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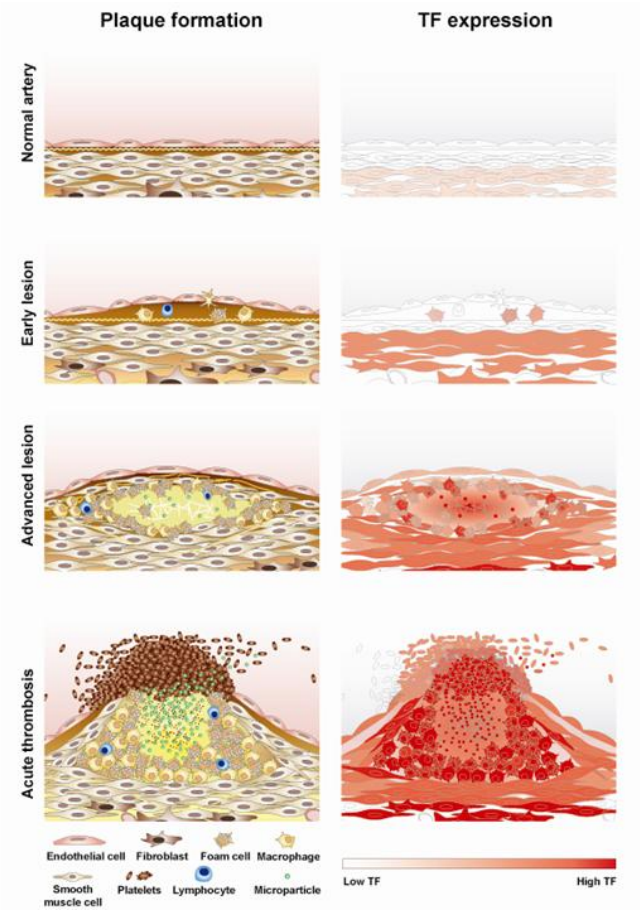
Plaque formation
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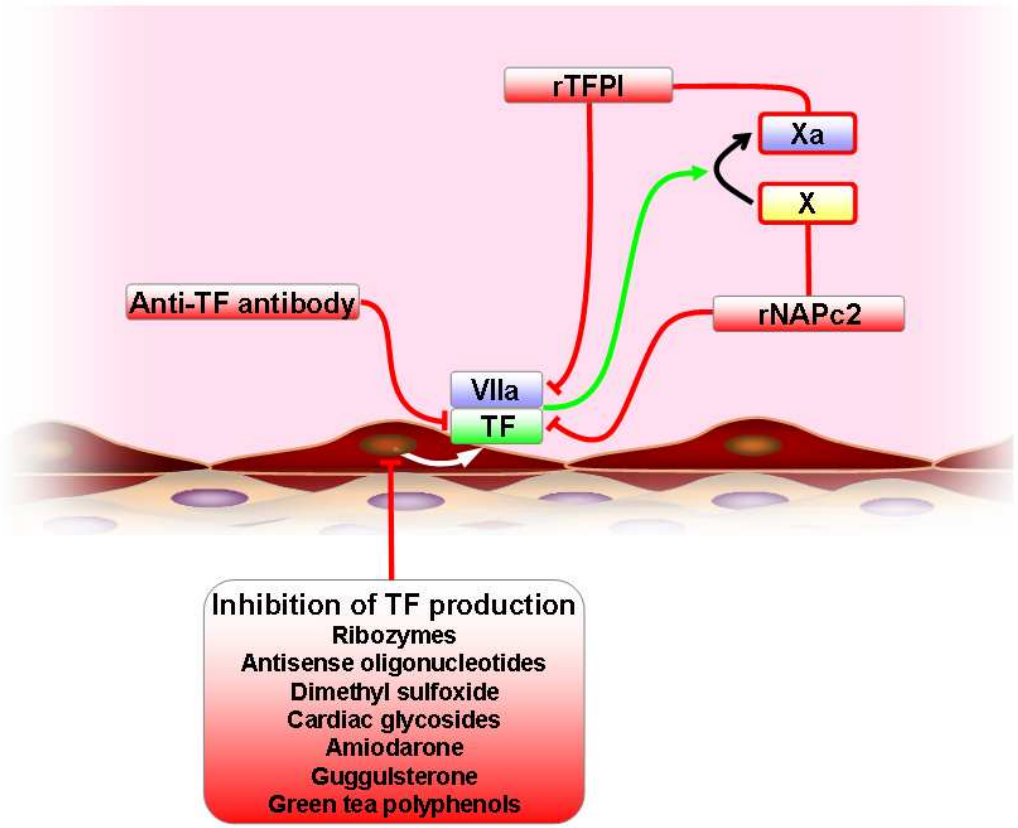
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Guggulsterone
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