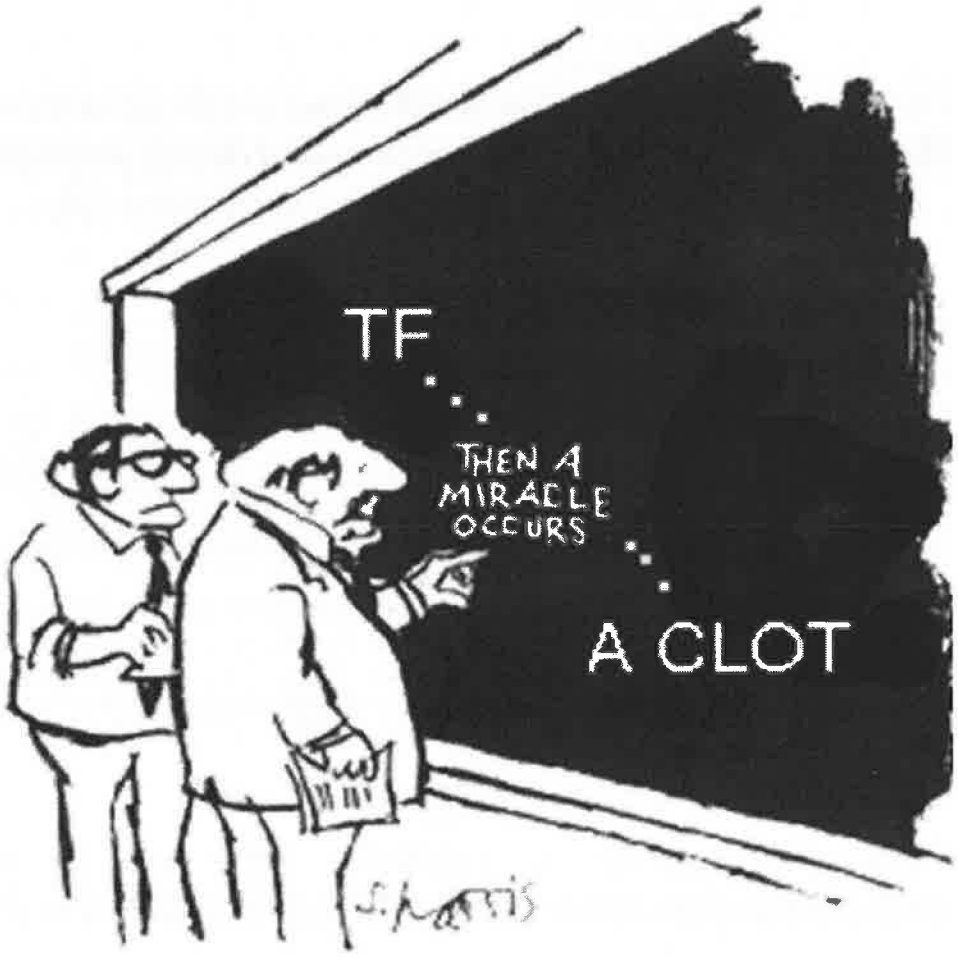
Tissue Factor:

Getting to Know an Old Friend



"I think you should be more explicit here in step two."

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INTERNAL MEDICINE GRAND ROUNDS
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER
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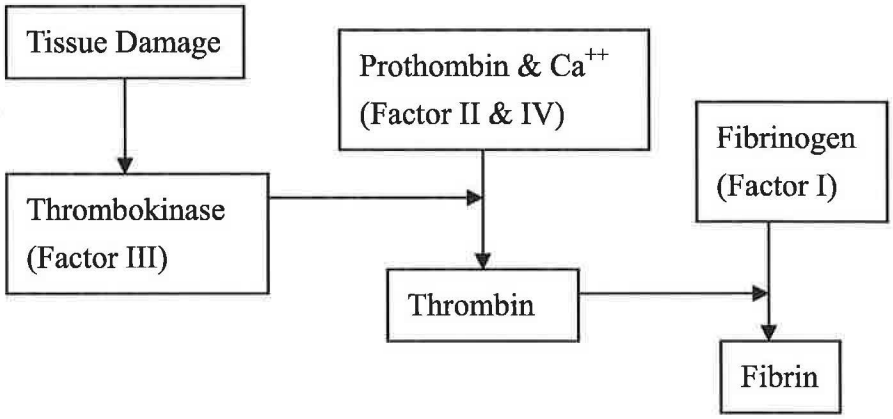
This is to acknowledge that Dr. Shen has disclosed any financial interests or other relationships with commercial concerns related directly to this program. Dr. Shen will be discussing off-label uses in his presentation.

The Historical Perspective

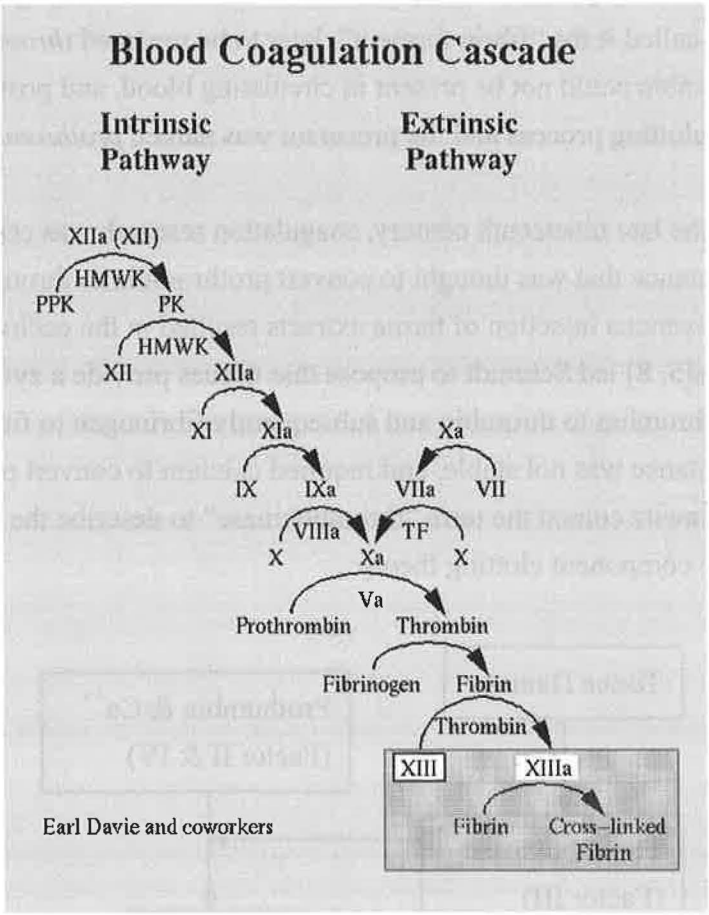
Coagulation of blood is a fascinating process that has mystified researchers since the beginnings of science. Plato believed that "fibers" in the blood aided clotting as the blood cooled[1]. Aristotle thought the fibers were made of earth, and blood without the fiber did not clot[2]. Little was known about the source of the fiber until the 1770s when Hewson localized the source to what we now call plasma[3]. Virchow and others in the mid 1800s established that *fibrinogen* is the precursor to *fibrin*, the fibers described by the ancient philosophers[4]. Buchanan[5] and Schmidt[6] discovered the converting agent that turned fibrinogen to fibrin, and called it the "fibrin ferment", later to be renamed *thrombin*. Schmidt realized that thrombin could not be present in circulating blood, and postulated that thrombin is a product of the clotting process and the precursor was named *prothrombin* by Pekelharing[7].

By the late nineteenth century, coagulation research was centered on the clot-promoting substance that was thought to convert prothrombin to thrombin. Observations that intravenous injection of tissue extracts resulted in the occlusion of animal blood vessels with clots[5, 8] led Schmidt to propose that tissues provide a zymoplastic substance which converts prothrombin to thrombin and subsequently fibrinogen to fibrin. The tissue clot-promoting substance was not stable, and required calcium to convert prothrombin to thrombin[9]. Morawitz coined the term "thrombokinase" to describe the clot-promoting substance in his four-component clotting theory:



Various terms were used to refer to the initial activator of coagulation. Nolf introduced the term "thromboplastin,"[10] and Howell introduced the term "tissue factor" in 1935[11]. Armed with the clotting theory of Morawitz, Quick developed the quantitative prothrombin test (PT) [12]. The PT became widely used in clinical practice to monitor oral anticoagulation, introduced at about the same time. There were test results that could not be explained by the four-component clotting theory, and patients with significant bleeding tendency but normal PT

or abnormal PT were reported. With mixing studies using patient plasma, various factors were discovered[13-16]. By 1962, 12 distinct clotting factors were described, and the classical theory of Morawitz was no longer adequate. Two thromboplastins were invoked: the *plasma thromboplastin* to initiate the intrinsic system, and the *tissue thromboplastin* to activate the extrinsic system. Plasma thromboplastin was active when plasma came into contact with foreign surfaces and was thought to interact with factors VIII, V, IX, XI, X and XII and calcium, platelets or phospholipids. Tissue thromboplastin was thought to be a lipoprotein that interacted with factors VII, X and V, prothrombin and calcium[17].



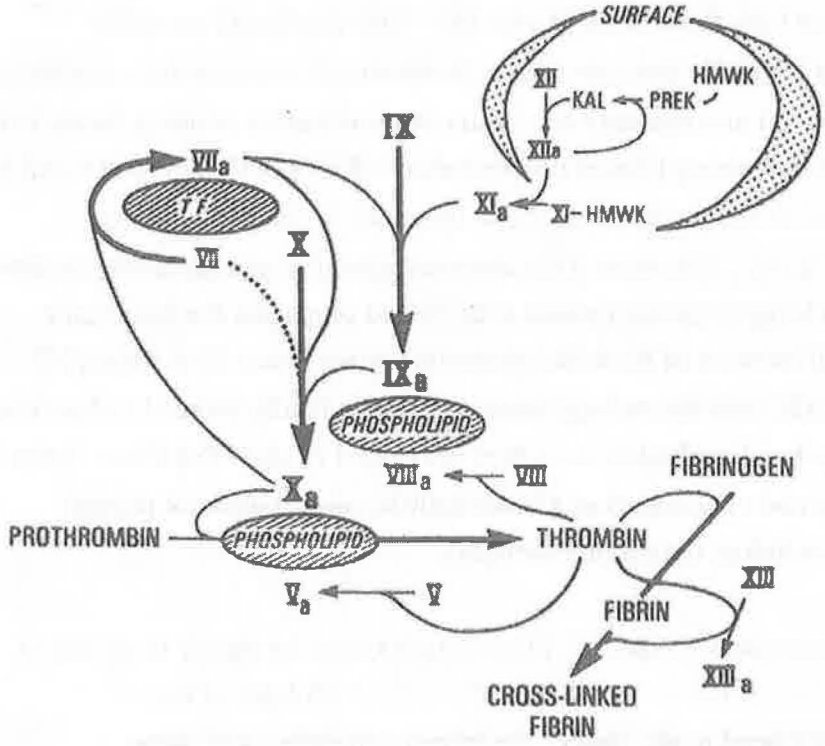
Thus came the "waterfall" or "cascade" theory of coagulation, which put the spotlight on the intrinsic (contact) system, with the activation of factor XII at the top of the cascade of *in vivo* coagulation[18, 19]. The nature of tissue factor was still unknown. No human disease could be attributable to tissue factor deficiency, while all other coagulation factors were described from distinct deficiency states in patients. Patients with hemophilia A and B had normal PT, but clearly abnormal "contact activation" with a prolonged partial thromboplastin time (PTT), performed by replacing the tissue thromboplastin used in PT with dilute tissue thromboplastin or crude cephalin (phospholipids from brain or spinal cord tissue)[20].

However, the waterfall theory could not explain certain observations. Patients with factor XII or Hageman factor deficiency have markedly prolonged PTT but do not have a bleeding tendency. Factor XI deficient patients have only a mild bleeding diathesis, with bleeding mainly after surgery, and rarely after trauma into tissues. On the other hand, factor VII deficient patients have a significant bleeding tendency[21]. These observations led to the conclusion that contact activation of the intrinsic system may not be the main initiator of coagulation *in vivo*.

In 1977 a crucial discovery was reported by Rapaport and Osterud, [22] who showed that tissue factor and factor VII activated both factor X and factor IX. This confirmed an earlier observation by Rapaport that factor IX was activated in disseminated intravascular coagulation when tissue extracts was injected into rabbits[23]. Mann showed that by omitting factor VIII or IX from a reaction system containing plasma concentrations of factors II, V, VIII, IX and X, thrombin generation was diminished to one third of that observed in reaction systems containing factor VIII and IX[24]. The same study demonstrated multiple feedback reactions (activation of factor V and VIII by thrombin) which amplify and propagate the hemostatic response, and only a minimal increase of thrombin generation when factor XI is added[24]. With the advancement of purification technology, tissue factor was finally isolated and purified in the 1980s[25-27]. Monoclonal antibodies were then developed to show that tissue factor is present around blood vessels and vital organs as a hemostatic envelop, but is not present normally on vascular cells, including the endothelium[28].

Tissue factor or the tissue factor-factor VIIa (TF-VIIa) complex must be tightly regulated to avoid unbridled activation of coagulation. Evidence of a natural inhibitor of tissue factor-initiated coagulation surfaced in the 1940s. In animal experiments of tissue factor-induced disseminated intravascular coagulation using placental extract, pre-incubation of the placental extract with serum prevented the death of the animals[29, 30]. The target of the putative inhibitor was shown to be the TF-VIIa complex, rather than tissue factor alone[31]. The inhibition was later demonstrated to be factor Xa dependent[32]. Two separate groups named it liporprotein-associated coagulation inhibitor and extrinsic pathway inhibitor. In 1991, it was renamed tissue factor pathway inhibitor (TFPI). TFPI was shown to first complex with factor Xa, then form a quarternary complex with the TF-VIIa complex to inhibit factor VIIa[33].

Thus the tissue factor pathway of coagulation is defined as follows: upon attaching to its membrane-bound cofactor/receptor tissue factor, factor VII is activated to VIIa. The TF-VIIa complex then activates factor X and factor IX. Only a trace amount of thrombin is generated from factor Xa, as this initiation phase is quickly inhibited by TFPI. In a positive feedback manner, thrombin activates factors V, VIII, IX and XI, generating enough thrombin to form a fibrin clot independent of TF-VIIa. Thus after nearly a century of coagulation research and introduction of the concept, tissue factor finally became the "prima ballerina" in the initiation of coagulation during hemostasis[33, 34].



From Rapaport & Rao, *Thrombosis and Haemostasis* 1995[34]

The role of platelets in hemostasis is by itself a subject for IM Grand Rounds. Briefly, Zahn had observed that bleeding from an injury to a blood vessel was initially blocked not by fibrin, but by a "white thrombus."[35] Several investigators had observed a colorless corpuscle in blood that was smaller than red cells or white cells. Bizzozero and Hayem showed that fibrin was associated with these cells, which led them to conclude that platelets supply a factor that is required for coagulation[36, 37]. Others later showed that the rate of clotting and prothrombin consumption was low in platelet-poor plasma and increased as a function of platelet number[38-40]. These early observations established the importance of both platelets and plasma proteins in coagulation, that platelets formed the initial hemostatic plug, and fibrin stabilized the platelet plug. It was assumed that membrane phospholipid, specifically phosphatidylserine, was the major determinant of platelet coagulant activity. Due to the difficulty in the preparation of platelet suspension, it was recommended to use a chloroform extract of brain (lipids) in clotting assays instead of platelets[41]. The use phospholipid vesicles allowed biochemical studies of the coagulation proteins to be done more easily and reproducibly. Clinical laboratory tests were likewise made reproducible.

Up to this point, coagulation research employed proteins and phospholipids in suspension to mimic *in vivo* events. However, this was naturally artificial, even though the acellular system provided reproducibility and advanced our understanding of the hemostatic process, which up

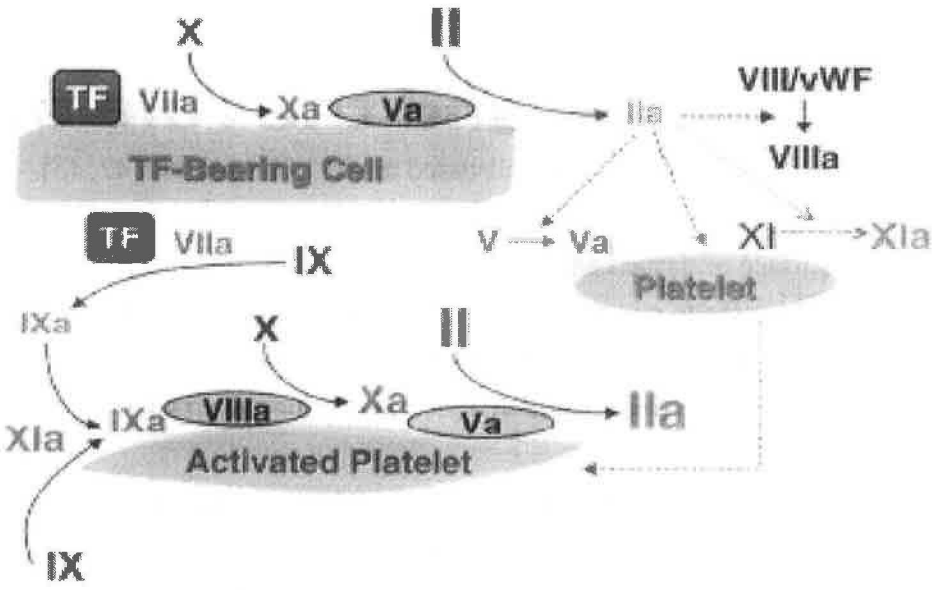
to this point had not taken into account the contribution of cellular elements other than platelets. In the 1980s Hoffman and Roberts hypothesized that putting real live cells back in the conceptual and experimental models of coagulation might more closely reflect hemostasis *in vivo*. Their research using monocytes as a source of tissue factor and unactivated platelets as a source of phospholipids led to the current cell-based model of hemostasis[42, 43].

1. Initiation: On the surface of tissue factor bearing cells, the combination of tissue factor and factor VII upon exposure to circulating blood leads to the direct activation of factor X to Xa. The reaction is not robust and can be effectively inhibited by TFPI. The combination of TF and FVII also is capable of activating factor IX to IXa.

2. Amplification: The small amount of factor Xa produced by TF-VIIa interaction leads to a limited amount of thrombin generation. The amount of thrombin produced is inadequate to support normal fibrin generation and in fact can be significantly inhibited by antithrombin. The signal becomes amplified when thrombin binds to platelets and initiates several positive feedback loops. The "priming action" of thrombin includes the activation of factor V to Va, which assembles into the prothrombinase complex with factor Xa on the activated platelet membrane; the release of free factor VIII from von Willebrand factor, resulting in factor VIIIa, which assembles into the tenase complex with factor IXa; conversion of factor XI to XIa, which generates more factor IXa; and the activation of platelets. Thus the stage is set for the thrombin burst, essential for stable clot formation.

3. Propagation: The assembled enzyme complexes on the activated platelet surface rapidly lead to the production of enough thrombin to support additional platelet activation. Additional platelet activation leads to ever-increasing amounts of thrombin and subsequent fibrin formation. The platelet produced thrombin has multiple actions in addition to clotting fibrinogen. It also stabilizes the clot by activating factor XIII & thrombin-activated fibrinolysis inhibitor.

4. Termination: As the site of coagulation initiation is "paved over" by deposition of platelets and fibrin, the activated clotting factors formed at the site cannot diffuse through the overlying layer of clot. Thus the sequestration of activated factors within the hemostatic clot may be enough to appropriately shut off thrombin generation. The small amounts of thrombin that may escape into circulation will be inactivated by circulating antithrombin, and thrombomodulin on the surface of normal endothelium will turn thrombin into an anticoagulant by activating the protein C pathway, inactivating factor Va and VIIIa.



From Hoffman, Monroe & Roberts, *Blood Coagulation and Fibrinolysis* 1998[44]

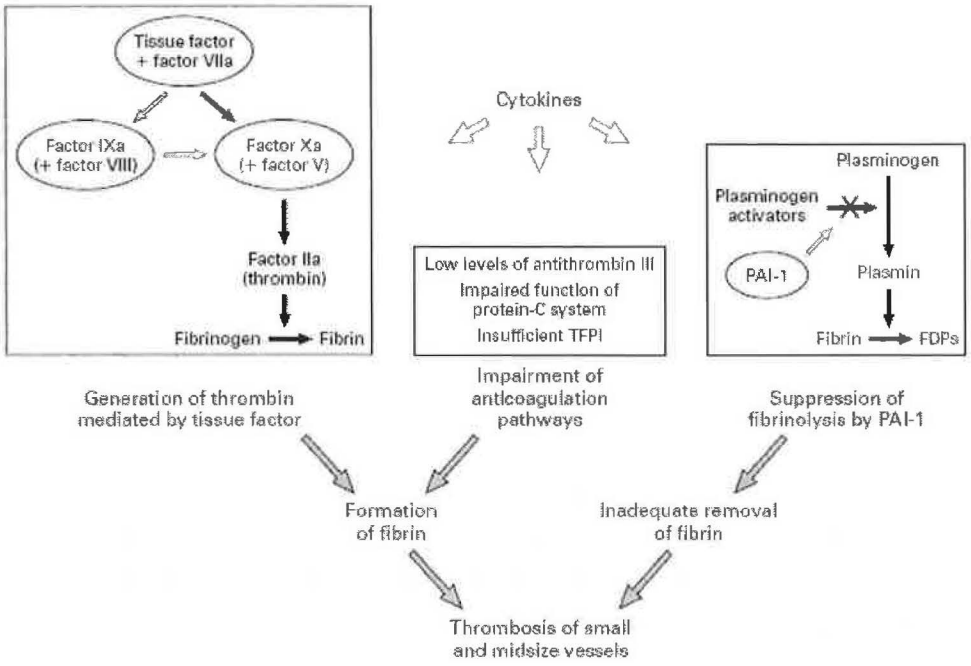
The cell-based model of hemostasis is believed to reflect more closely what occurs in vivo. The classical cascade still applies in that the extrinsic pathway occurs on the tissue factor bearing cells and is assessed by PT, and the intrinsic pathway occurs on the surface of platelet and is assessed by PTT. In our most advanced understanding of hemostasis, tissue factor remains as the initiator of the coagulation process, with the final product thrombin as the major effector of hemostasis.

Since tissue factor assumed the central role in coagulation, it has been implicated in the inflammatory process as well as tumor biology. The discovery and elucidation of the new functions of tissue factor mark the beginning of a new era in coagulation research.

Role of Tissue Factor in Inflammation

It is familiar to clinicians of all fields that the systemic inflammatory response brought about by infection, trauma, or obstetric complications is often complicated by disseminated intravascular coagulation (DIC). This is often associated with multi-organ failure and high mortality rates[45]. As our understanding of coagulation and inflammation advanced, it is increasingly clear that there is extensive cross-talk between the two processes[46]. Activation of coagulation and subsequent fibrin deposition as a result of inflammation can be thought of as part of the normal host defense system to contain the invading entity and contain the inflammation to a limited area. In endotoxemia, the inflammatory cytokines orchestrate an

imbalance between tissue factor-mediated fibrin deposition and antithrombin/protein C-mediated natural anticoagulant processes[47], as well as defective fibrinolysis[48]. The end result is DIC, an exaggerated and/or poorly controlled response to systemic inflammation that contributes to the pathologic process[45].



From Levi and ten Cate, *NEJM* 1999[45]

The role of inflammatory cytokines in the induction of coagulation during endotoxemia has been an area of active investigations. Exogenous infusion of recombinant tumor necrosis factor (TNF) in healthy human volunteers or cancer patients activated the coagulation system identical to that induced by endotoxin[49, 50]. However, anti-TNF monoclonal antibody could not abrogate the activation of coagulation after endotoxin infusion, but it was able to completely eliminate the increase in endogenous TNF in primates[51, 52]. Treatment of septic patients with anti-TNF monoclonal antibody was also not beneficial in some of the early trials[53]. In contrast, studies of interleukin-6 (IL-6) do suggest that it is a relevant mediator of procoagulant induction in DIC. Thrombin generation was observed after infusion of recombinant IL-6 in cancer patients[54], and anti-IL-6 monoclonal antibody completely blocked the endotoxin-induced activation of coagulation in chimpanzees[55].

Although TNF is not an important mediator of the initiation of coagulation in DIC, it appears to be the principal mediator in the suppression of the protein C system by down-regulating thrombomodulin on endothelial cells[56] (thrombomodulin is required for the activation of protein C by thrombin). TNF is also implicated in the suppression of fibrinolysis as a result of increased plasminogen activator inhibitor-I (PAI-1)[48].

The principal initiator of inflammation-induced thrombin generation is tissue factor. Blocking tissue factor activity will completely abrogate the induction of coagulation in experimental endotoxemia or bacteremia,[57] whereas antibodies that inhibit factor XII had no effect on thrombin generation[58], and antibodies that block factor VIII had no effect on fibrinogen consumption[59]. In sepsis, the lungs, kidneys and liver are more sensitive to dysfunction and subsequent failure[60]. In the lungs and kidneys, this appears to be related to the over-expression of tissue factor as shown by studies in various animal models of endotoxemia[61-63]. Tissue factor expression was also induced in monocytes and in a subset of endothelial cells in endotoxemia and sepsis, under the influence of inflammatory cells and cytokines[62, 63]. Increased levels of "blood-borne" tissue factor were also observed[64]. An imbalance between tissue factor and its natural inhibitor TFPI also exists in sepsis, likely contributing to the development of DIC and poor outcome[65, 66]. Thus during the pathological condition of systemic inflammation, tissue factor expression can be induced in the intravascular space, contributing to activation of coagulation and fibrin deposition.

Inhibition of the TF-VIIa complex by various agents (TFPI, monoclonal anti-TF antibody, active site inhibited factor VIIa) in animal models reduced the risk of endotoxin-induced coagulation, inflammatory response, and mortality[67-71]. Mice lacking tissue factor on their hematopoietic stem cells were less susceptible to inflammation[72]. Experiments using DEGR-Xa, an active site-modified, inactive derivative of factor Xa, in endotoxemia completely blocked the coagulation response but did not alter lethality[73]. Recombinant nematode anticoagulant protein c2 (rNAPc2), a potent factor Xa dependent inhibitor of the TF-VIIa complex, blocked the procoagulant response but not the pro-inflammatory or anti-fibrinolytic response[74, 75]. Presumably this was a result of continued factor Xa activity despite rNAPc2 binding. These results suggest that the initial coagulation complex of tissue factor and factor VIIa has pro-inflammatory effects that are independent of the procoagulant effects. Indeed, the administration of recombinant factor VIIa to healthy human volunteers caused a small but significant increase in plasma IL-6 and IL-8[76].

As mentioned above, there is a cytokine-mediated imbalance between the procoagulant actions of tissue factor and the anticoagulant actions of the protein C system in sepsis. Activated protein C, with the help of its endothelial protein C receptor on the endothelial cells, has anti-inflammatory effects to counteract the pro-inflammatory process seen in sepsis[77, 78]. Although the molecular mechanism of the anti-inflammatory effects of activated protein C is to be clarified, evidence exists to suggest that protease activated receptors are involved[79]. This is the basis for the beneficial effects of activated protein C (drotrecogin alfa) infusion in severe sepsis[80].

Role of Tissue Factor in Tumor Biology

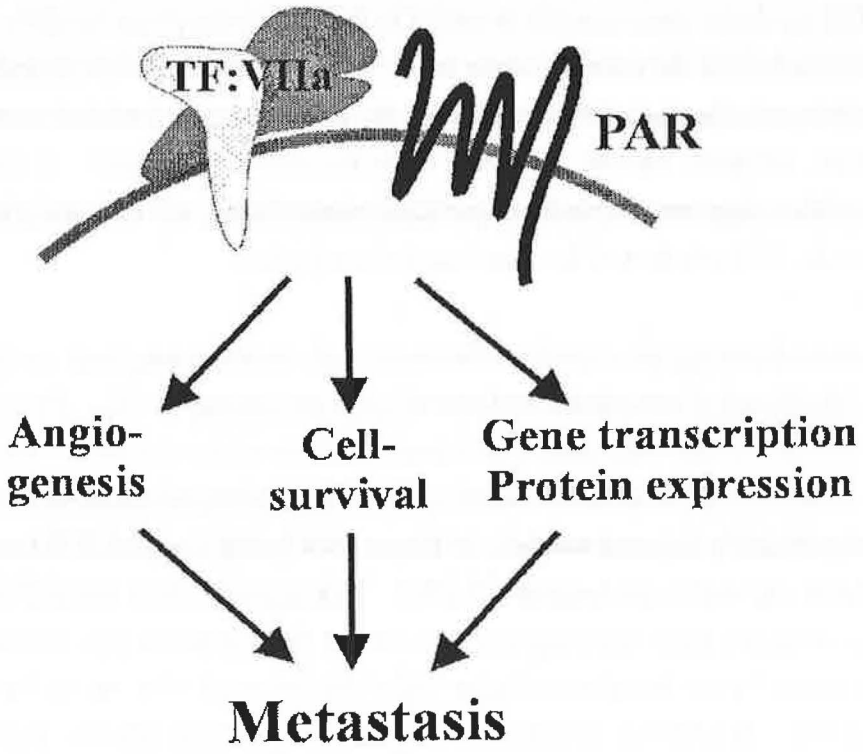
Venous thromboembolism and DIC are frequent complications of malignancy referred to as Trousseau's syndrome, first described by Trousseau in 1861[81]. Various mechanisms have been described from direct vascular compression to co-existence of thrombophilia such as factor V Leiden or antiphospholipid antibodies/lupus anticoagulant. Rapaport and Callander showed that patients with Trousseau's syndrome have persistent low-grade DIC, and provided pathological evidence that tumor cells express surface tissue factor and the continuous exposure of tumor associated tissue factor to circulating blood is likely to initiate the coagulation process[82]. Increased expression of tissue factor has been documented in cancers of the breast, colon, pancreas, lung, head and neck, urothelium and glioma[83-86]. The tissue factor expressed by tumor cells is active as a procoagulant and is able to form fibrin clot if given the proper coagulation factors and conditions[87].

Metastasis is the development of tumors at secondary sites remote from the primary site. A number of physiologic processes are involved in the metastatic phenotype[88, 89]. Cancer cells need to detach from the primary tumor mass, migrate toward the lymph and/or blood vessels, penetrate into the vascular lumen, evade the immune system while in transit, adhere to the endothelium, infiltrate, survive, and grow in the invaded environment. It is now clear that components of the coagulation system, in particular tissue factor, are intimately involved in the metastatic process independent of the procoagulant properties.

First of all, tissue factor expression often correlated with tumor grade, stage of disease progression, likelihood of metastasis, and overall poor prognosis[90-96]. Over-expression of tissue factor was associated with enhanced tumor cell invasion *in vitro*[97]. Mueller showed in 1992 that inhibition of tissue factor function abolished prolonged adherence of tumor cells resulting in significantly reduced numbers of tumor cells in the lungs of SCID mice injected with tissue factor expressing melanoma cells[98]. In a separate study using Chinese Hamster Ovary (CHO) cells, the same investigators showed that the metastatic potential was not only dependent on tissue factor, but also on factor VIIa proteolytic activity; factor Xa involvement was excluded[99]. In addition, transfection of CHO cells with cytoplasmic domain-deleted tissue factor resulted in poor metastasis in SCID mice, indicating the metastatic promoting activity of tissue factor is also dependent on the intracellular tail of tissue factor.[99] Furthermore, inhibition of the TF-VIIa-Xa complex by TFPI injection or TFPI gene transfer reduced experimental lung metastasis of B16 melanoma cells[100].

In order for the metastatic tumor cells to survive at the invaded location, new blood vessels must develop to support the new growth. Tissue factor has a critical impact on both

embryonic and tumor vessel formation. No tissue factor knock-out mice survived beyond E10.5; there was catastrophic yolk sac cavity hemorrhage between E8.5 and E9.5 associated with defective vascular development.[101, 102] Thus tissue factor plays an indispensable role in establishing and/or maintaining vascular integrity in the developing embryo. Similarly, both clinical and experimental studies have demonstrated the role of tissue factor in tumor angiogenesis. A correlation between tissue factor expression and microvessel density was observed in non-small cell lung cancer[103] and prostate cancer[104]. In addition, there was a significant correlation between tissue factor and vascular endothelial growth factor (VEGF) expression in certain human tumors[103, 105, 106]. Importantly, over-expression of tissue factor also appeared to be associated with a switch in the angiogenic balance toward a more pro-angiogenic phenotype with up-regulation of VEGF and down-regulation of the anti-angiogenic thrombospondin (TSP)[107].

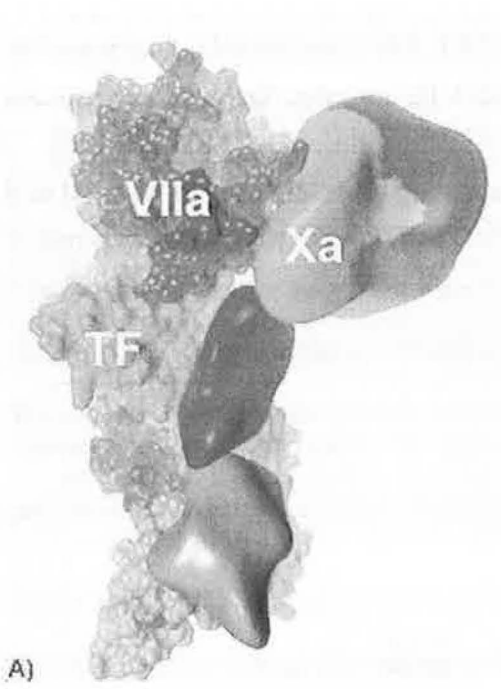


From Versteeg, et al, *Molecular Medicine* 2004[108]

Metastatic tumor cells often survive by inhibition of apoptosis. Given the intimate relationship of tissue factor in the metastatic process described above, it is perhaps not surprising that the TF-VIIa complex formation is associated with activation of the anti-apoptotic pathways. Factor VIIa was capable of inducing cell survival in BHK and CHO

cells over-expressing tissue factor, by potently reversing serum starvation-induced apoptotic changes and activation of caspase-3[109]. Even more relevant to the metastatic process is the reversal of anoikis, a special form of apoptosis that occurs when cells detach from the extracellular matrix and thus lack adhesion signaling[110]. Thus it appears that TF-FVIIa has downstream effects to replace the adhesion signaling, leading to aberrant cell survival.

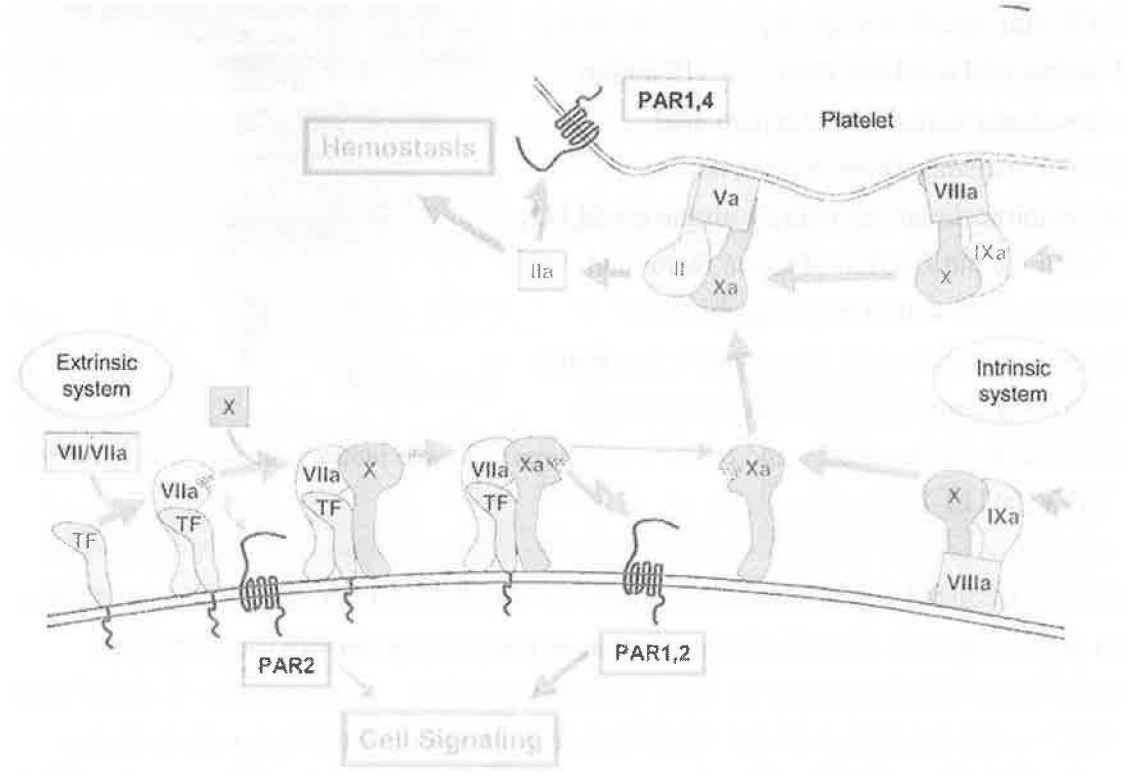
Tissue Factor and Intracellular Signaling



In order for tissue factor and the initial coagulation complex between tissue factor and factor VIIa to promote inflammation in sepsis and mediate the metastatic phenotype in tumor cells, there has to be cellular processes that are initiated by tissue factor or TF-VIIa, i.e., signal transduction. Tissue factor is a 47 kDa transmembrane glycoprotein consisting of 263 amino acid residues; there is a 219 amino acid extracellular domain, a 23 amino acid hydrophobic transmembrane region, and a C-terminal intracellular tail with 21 amino acids[111, 112]. With the purification of tissue factor and identification of its amino acid sequence and three-dimensional structure, tissue factor was found to have striking homology with interferon γ-type receptors both in the secondary and tertiary structures. Tissue factor is in fact a receptor for factor VII/VIIa[113-115].

Tissue factor mediated signal transduction occur by two distinct but related routes, vertically through the cytoplasmic domain of tissue factor, or horizontally through the proteolytic function of factor VIIa to activate protease activated receptors. The cytoplasmic tail of tissue factor has 3 serine residues, 2 of which can be readily phosphorylated by a protein kinase C-dependent mechanism[116]. The phosphorylated serine residues then serve as potential docking sites for signaling proteins. The cytoplasmic tail also appears to be essential for the generation of calcium transients[117], tissue factor mediated expression of VEGF[118], and metastasis[99]. In addition, the cytoplasmic tail has a high-affinity binding site for actin-binding protein-280, implicating involvement in cell adhesion and migration[119].

The horizontal signal transduction is dependent on the activation of a set of receptors referred to as the protease activated receptors. This is dependent on the extracellular proteolytic activities of the coagulation proteases generated by tissue factor, namely, factor VIIa, factor Xa, and thrombin. Protease activated receptors (PARs) belong to the large protein family of seven transmembrane domain, G-protein-coupled receptors. The PARs are predominantly activated by trypsin-like serine proteases that recognize and cleave a specific arginyl peptide bond in the amino-terminal ectodomain of the receptor, leading to the exposure of a neo-amino terminus that folds back and activates the same or possibly an adjacent receptor[120-122]. In other words, the PARs do not bind a signal-transducing ligand, but rather recognize their own, tethered ligand that is exposed by proteolytic cleavage. Of the four known human PARs, PAR1, PAR3 and PAR4 are activated by thrombin, whereas PAR2 can be activated by the TF-VIIa complex, factor Xa, trypsin and mast cell tryptase[123]. Factor Xa also activates PAR1 in addition to PAR2[124-126]. Riewald and Ruf showed in 2001 that protease signaling of tissue factor is coupled to the initiation of coagulation, i.e., signaling is integral to the TF-VIIa initiated coagulation pathway[127].



From Riewald and Ruf, *TCM* 2002[123]

Classical signal transduction pathways are activated in tissue factor signaling. Experiments in various cell types, including human endothelial cells induced to express tissue factor, demonstrated transient cytosolic calcium oscillations induced by factor VIIa[128]. The calcium signal was critically dependent on the proteolytic activity of factor VIIa and

phosphatidyl inositol-specific phospholipase C[129]. Depending on the cell used in the experimental model, the TF-VIIa dependent calcium signal may or may not be dependent on the cytoplasmic tail of tissue factor[125, 130].

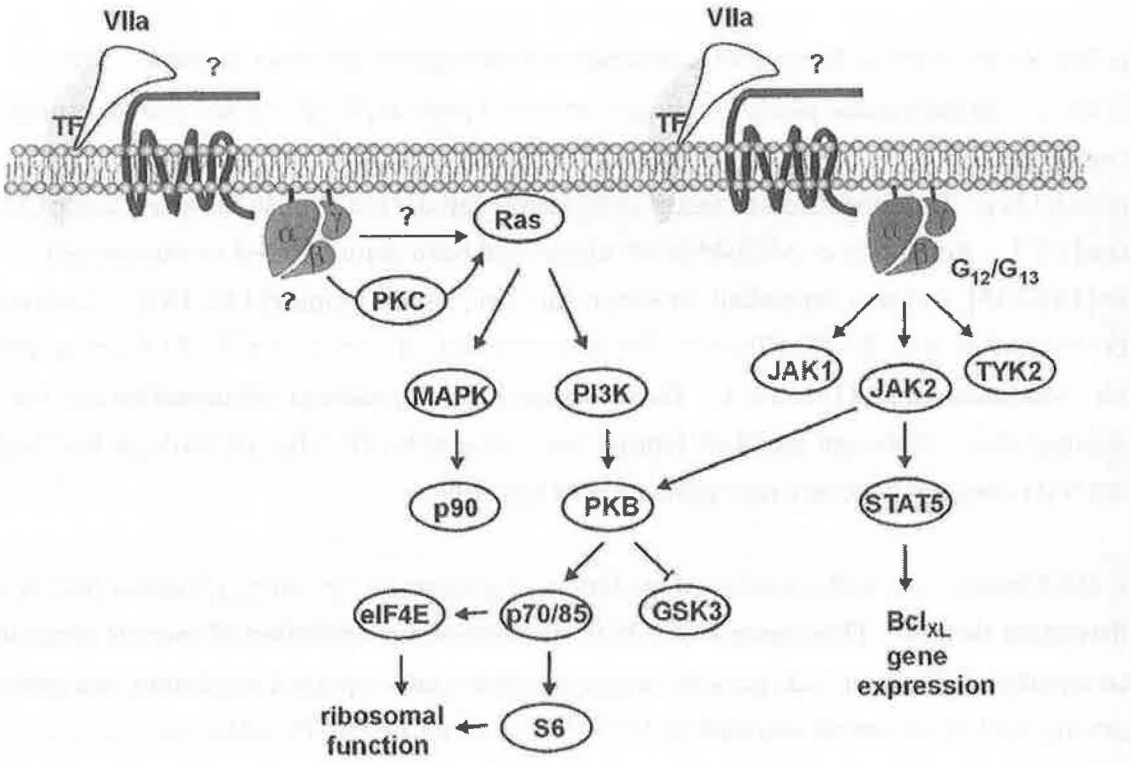
The first report of tissue factor-FVIIa-dependent kinase activation came in 1998. The activation of promitogenic p42/p44 mitogen activated protein (MAP) kinase was dependent on the upstream activation of MEK kinase, and functional factor VIIa was absolutely required[131]. Deletion of tissue factor cytoplasmic tail did not abolish the activation of MAP kinase[132]. Activation of p42/p44 MAP kinase had been demonstrated in various cell types[133-136], and was dependent on Ras, c-Raf, Src, and PI3-kinase[136, 137]. Activation of other MAP kinases by TF-VIIa was also demonstrated, in particular p38 MAP kinase and c-Jun N-terminal kinase[133, 136]. These kinases have key roles in inflammation and the stress response. Although the MAP kinases are activated by TF-VIIa, physiologic levels of factor VIIa does not have any mitogenic effects[138, 139].

The MAP kinases are well-established mediators of gene transcription by phosphorylation of transcription factors. Thus, upon factor VIIa stimulation, up-regulation of specific genes had been reported. These include growth factors, cytokine transcriptional regulators, and genes regulating cell organization and motility[140, 141]. In particular, fibroblast growth factor-5, heparin-based epidermal growth factor, connective tissue growth factor, and Cyr61 mRNA transcriptions were demonstrated to increase with factor VIIa stimulation of fibroblasts and immortalized keratinocytes (HaCaT) [140, 141]. The latter two are extracellular matrix signaling proteins that facilitate angiogenesis. Inflammatory cytokine genes *IL-1β, IL-8, MIP2a,* and *LIF* were up-regulated by factor VIIa stimulation in keratinocytes[140]. Importantly, IL-1β and IL-8 are strong inducers of angiogenesis.

Tissue factor-FVIIa induced protein synthesis was described recently. Physiological concentrations of factor VIIa promote protein synthesis by activation of S6 kinase phosphorylation, which activates the ribosome and up-regulate eukaryotic elongation factors eEF1a and eEF2. The enhanced protein synthesis was independent of the cytoplasmic domain as well as factor Xa or thrombin generation, and may well contribute to the TF-VIIa effects in pathophysiology.[142]

To summarize, there is now ample evidence that tissue factor, the primary initiator of the coagulation process, is intimately involved in inflammation and tumor metastasis. Although downstream coagulation proteases, factor Xa and thrombin, may exert a similar influence, the TF-VIIa complex plays a direct role through activation of signal transduction pathways via the proteolytic cleavage of PARs and the phosphorylation of the cytoplasmic tail of tissue factor.

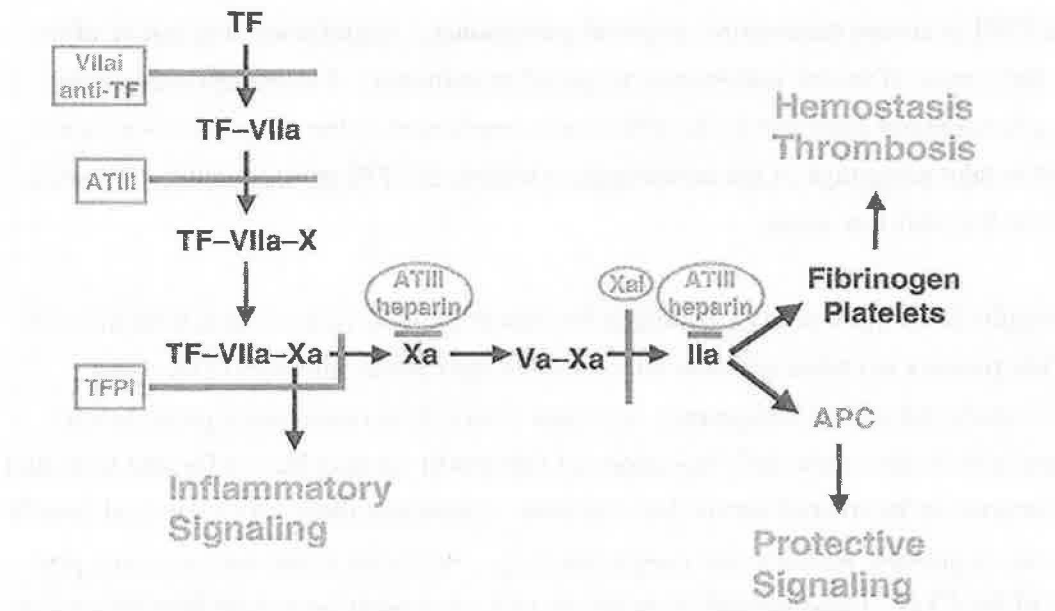
Investigations of the underlying mechanisms are particularly relevant for the potential clinical use of coagulation inhibitors in sepsis and cancer therapy.



From Versteeg and Ruf, *Seminars in Thrombosis and Hemostasis* 2006[143]

Targeting Tissue Factor

Knowledge of the mechanisms by which the procoagulant and anticoagulant pathways regulate the inflammatory balance *in vivo* provides a new approach to anticoagulant therapy in sepsis. Preclinical studies mentioned in this presentation support the use of agents that target the tissue factor initiation complex are expected to have anti-inflammatory benefits that are not possible with agents that target thrombin. Antithrombin, the natural circulating thrombin inhibitor, can also inhibit TF-VIIa. In preclinical sepsis models recombinant antithrombin attenuateed the inflammatory response and improved survival, similar to other direct tissue factor inhibitors[144].



From Riewald and Ruf, *Critical Care* 2003[145]

The KyberSept Trial, a randomized placebo-controlled phase 3 trial using high dose recombinant antithrombin, demonstrated no effect on 28-day all cause mortality in patients with severe sepsis. There was some evidence to suggest a treatment benefit in the subgroup of patients who did not receive concomitant heparin[146]. Heparin will displace antithrombin from cell surfaces where coagulation signaling complexes are expressed, and enhance the inhibitory activity against fluid-phase thrombin. The reduction in thrombin-dependent activated protein C generation and subsequent APC-mediated protective signaling also obviates any beneficial effects from decreased fibrin deposition[145].

The OPTIMIST Trial, a randomized placebo-controlled phase 3 trial using recombinant TFPI (Tifacogin) in patients with severe sepsis and high INR, demonstrated no effect on all-cause mortality[147]. Heparin releases TFPI from endothelial cells. Thus any beneficial effect from TFPI might have been negated by the non-random use of heparin. The inclusion of patients with high INR selected patients with more severe disease.[148] In addition, there was laboratory evidence that TF-FVIIa-FXa complex dependent activation of PAR1 remained intact when tissue factor mediated Xa generation was blocked with TFPI, suggesting that TFPI may not inhibit tissue factor-induced signaling[149]. Earlier, a small human endotoxemia study also demonstrated that TFPI did not influence the inflammatory pathways, contrary to what was observed in animal models[150]. This may be explained by the fact that TFPI can only inhibit TF-VIIa after TFPI binds to factor Xa, a time lag that may be adequate to allow the pro-inflammatory events to occur.

CAPTIVATE is an on-going phase 3 study examining whether there is a beneficial effect of recombinant TFPI in severe community acquired pneumonia. Significant lung injury often complicates the course of severe community acquired pneumonia. Fibrin deposition from exaggerated procoagulant response to the infection is implicated in the pathogenetic process. The rationale to take advantage of the anticoagulant effects of TFPI is reasonable. We shall see the result in the next few years.

Tantalizing results from trials of anticoagulants for cancer patients indicate that inhibition of the coagulation process in cancer patients may improve the cancer outcome[151]. The FAMOUS (Fragmin Advanced Malignancy Outcome Study) trial randomized patients with advanced cancer to receive once daily injections of dalteparin versus placebo for one year, and found no difference in the overall survival at one year. However, there was a survival benefit in the subgroup of patients with a better prognosis[152]. A similar result was seen in a post hoc analysis of the CLOT (randomized Comparison of Low-molecular weight heparin versus Oral anticoagulant Therapy) study[153], in that patients with no metastatic disease at the time of venous thromboembolism diagnosis had better survival with dalteparin[154]. A smaller study using nadroparin in patients with metastatic solid tumors demonstrated a small but statistically significant survival benefit compared to placebo[155].

With our knowledge that the pro-metastatic/pro-angiogenic effects of tissue factor are exerted at the initiation complex, it is no wonder that general anticoagulants that interfere with downstream coagulation proteases, i.e., heparins and vitamin K antagonists, are destined to fail as anti-cancer therapy. A rational target would be tissue factor. Given the complex, over-lapping oncogenic mechanisms, targeting tissue factor alone is likely not going to be beneficial by itself. This is a problem that complicates any targeted therapy today. Thus, the role of anti-tissue factor therapy is likely to be an adjunctive one, given in combination with traditional chemotherapy or with other targeted agents.

Thus far there has been no human trial using monoclonal antibodies against tissue factor or factor VIIa, or active site-inhibited factor VIIa, in either sepsis or cancer patients. New agents can be designed to have minimal anticoagulant effects to minimize bleeding seen with both the antithrombin and TFPI trials, but have potent anti-inflammatory/anti-metastatic effects by interfering with tissue factor signaling. Such novel agents could only come from a complete understanding of the molecular mechanism underlying tissue factor signaling and regulation of the hemostatic process. With our expanding knowledge of tissue factor in inflammation and tumor biology, the prima ballerina's repertoire has taken on surprising dimensions that will continue to fascinate many of us for years to come.

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