Introduction

Tissue factor (TF) is the major cellular initiator of blood coagulation (1, 2). Its primary function is to provide a haemostatic barrier around blood vessels by activating the coagulation protease cascade in the event of vascular injury (3). In contrast, aberrant TF expression contributes to thrombosis in a variety of diseases, including atherosclerosis, cancer and sepsis (4-6). Other studies have focused on TF-dependent signaling in vascular cells. This signaling is mediated by the activation of protease activated receptors (PARs) by the coagulation proteases FVIIa, FXa and thrombin (7-11). Recently, it has been shown that TF-dependent generation of coagulation proteases and PAR signaling contributes to various biological processes, including angiogenesis and inflammation (Fig. 1).

Tissue distribution of TF

TF expression levels vary in different organs. Brain, lung, heart, kidney, uterus, testis, skin and placenta express high levels of TF, whereas liver, spleen, skeletal muscle and thymus express low levels of TF (3, 12, 13). The cell types that express high levels of TF in these organs are astrocytes in the brain, cardiac myocytes in the heart, bronchiolar and alveolar epithelial cells in the lung and trophoblasts in the placenta (12, 14-17). The high level of TF in these organs may provide additional haemostatic protection (3, 14). Consistent with this concept, low TF mice (see below) exhibit spontaneous hemorrhages in the brain, lung, heart and in the uterus during pregnancy (15, 18, 19). Interestingly, the cell type-specific pattern of TF is similar in mice and humans in all tissues except the kidney (20). In humans, TF is expressed by glomerular cells, whereas these cells express low levels of TF to below a critical threshold that is required to maintain adequate haemostasis. Nevertheless, a reduction in TF:FVIIa-dependent signaling may also reduce cell survival and/or compromise the integrity of the vasculature in these organs. Low TF mice are also a useful tool to study the role of TF and the coagulation cascade in other processes, such as thrombosis, inflammation and angiogenesis.

Summary

Tissue factor (TF) contributes to a variety of biological processes by generating coagulation proteases and by the activation of protease activated receptors (PARs). Studies, using low TF mice, have provided us with novel insights into the role of TF in tissue-specific haemostasis. Low TF mice exhibit defects in the uterus, placenta, heart and lung, which are all tissues that normally express high levels of TF. We propose that these observed defects are primarily due to a reduction in the level of TF to below a critical threshold that is required to maintain adequate haemostasis. Nevertheless, a reduction in TF:FVIIa-dependent signaling may also reduce cell survival and/or compromise the integrity of the vasculature in these organs. Low TF mice are also a useful tool to study the role of TF and the coagulation cascade in other processes, such as thrombosis, inflammation and angiogenesis.

Keywords

Tissue factor, haemostasis, thrombosis, inflammation, angiogenesis

Role of tissue factor in haemostasis, thrombosis, angiogenesis and inflammation: lessons from low tissue factor mice

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cells do not express TF in mice (18, 21, 22). In low TF mice, the human TF promoter directs a “human pattern” of TF expression in the glomerular cells. The reason for this species-specific pattern of TF expression in the kidney is unclear.

**Generation and characterization of low TF mice**

TF deficiency results in intrauterine lethality, indicating an essential role of TF in survival (23-25). We rescued mouse TF deficient embryos with a minigene (hTF) containing the human TF promoter and cDNA (18). However, mice containing the transgene as the only source of TF (mTF+/hTF+) expressed very low levels of TF and were designated “low” TF mice. Quantification of the procoagulant activity in different tissues showed that low TF mice expressed TF at levels that were approximately 1-2% of wild-type levels (18). Immunohistochemical analysis of tissues from these mice demonstrated a cell type-specific pattern of human TF expression that was similar to that of mouse TF (18). Importantly, in low TF mice, human TF was expressed by adventitial cells surrounding blood vessels (Fig. 2).

Low TF mice had a prolonged tail bleeding time compared to wild-type mice (Fig. 3). We also observed that low TF mice were prone to excessive bleeding during major surgery. For instance, a stab wound injury to the brain caused the hemorrhagic death of all low TF mice within hours of surgery. In contrast, control mice with normal levels of TF recovered fully from a stab wound injury and showed no signs of hemorrhage. Finally, low TF mice exhibited reduced survival compared with wild-type mice (19). Autopsies revealed spontaneous hemorrhages in the lung, brain and intestine (18, 19). These results indicated that low TF mice have impaired haemostasis and exhibit excessive bleeding in the event of vascular injury.

**Role of TF in uterine haemostasis**

During pregnancy, implantation into and detachment of the placenta from the uterine wall are two processes that present major haemostatic challenges to the mother. This explains why the uterine epithelium expresses a high level of TF (15). We found that low TF mice exhibited a high incidence (14%-18%) of fatal postpartum hemorrhage when bred with either wild-type or low TF male mice (15). In contrast, mTF+/-/hTF+ female mice carrying both mTF+/- and low TF embryos rarely succumbed to fatal postpartum hemorrhages. These results indicate that low levels of TF in the uterus in low TF mice often failed to control bleeding after detachment of the placenta but provided adequate haemostasis during implantation. In contrast, female mice deficient in fibrinogen die of fatal hemorrhages during implantation at embryonic day (E) 10 due to impaired haemostasis and defective attachment of the placenta (26, 27). Importantly, female mice expressing low levels of mouse FVII also exhibit fatal hemorrhages during pregnancy (E. Rosen, pers. com.), indicating that the TF:FVIIa complex plays a critical role in uterine haemostasis. These results are consistent with the postpartum hemorrhaging in humans associated with deficiencies of coagulation proteins, such as FVII, FXIII and fibrinogen (28-30).

**Role of TF in the placenta**

The placenta is another tissue that expresses high levels of TF (31). We found that TF mRNA expression in the mouse placenta increased to a maximum level at E14.5 before decreasing (not shown). Importantly, the absolute volume and surface area of maternal blood spaces expands rapidly between E14.5 and E16.5 (32). Low TF females bred with low TF males had a high rate (42%) of fatal late-gestational hemorrhage (E13.5-E15.5) (15). In contrast, low TF female mice bred with wild-type males rarely had late-gestational hemorrhages. Interestingly, histological analysis of the placentas of these low TF embryos revealed maternal blood pools in the labyrinthine layer of the placenta between E13.5 and E15.5 that were not observed in wild-type placentas (Fig. 4) (15). This phenotype was dependent on the level of embryonically-derived TF, and was independent of maternally-derived TF. These studies suggest that embryonically-derived TF plays an important haemostatic role in the maintenance of the maternal blood spaces in the placental labyrinth at a time when these spaces are expanding rapidly.

These observations do not exclude the possibility that the TF:FVIIa-dependent generation of coagulation proteases, and subsequent PAR signaling, may also contribute to the maintenance of the placenta barrier. Indeed, low TF levels rescue the placental defect observed in thrombomodulin null embryos at E8.5 (33). This result suggests that TF and thrombomodulin may regulate local coagulation protease generation and PAR signaling in trophoblasts that is required for normal placental development (33).

**Hemosiderin deposition and fibrosis in hearts of low TF mice**

Cardiac muscle expresses high levels of TF, whereas skeletal muscle expresses very low levels of TF (3, 13, 16). We hypothesized that TF expression by cardiac myocytes provides additional haemostatic protection to limit hemorrhage in the heart (19). Young low TF mice (less than 2 months old) were healthy and did not exhibit any heart defects, indicating that TF was not playing a role in heart development. In contrast, the hearts of all older low TF mice contained hemosiderin deposits and areas of fibrosis (Fig. 5) (19). The extent of fibrosis increased with age. We found that cardiac fibrosis in low TF mice significantly impaired heart function (19). Hemodynamic studies revealed
that low TF mice had a marked reduction of heart contractility manifested by a significant decrease (30%) in left ventricular function. This decrease was not large enough to induce congestive heart failure, but we speculate that the extensive fibrosis may cause fatal arrhythmias in low TF mice. Similarly, low FVII mice had hemosiderin and fibrosis in their hearts (19). These results indicate that a major reduction in TF:FVIIa activity is associated with a selective defect in the heart.

The heart is an organ in which mechanical forces may injure blood vessels (34). Therefore, we proposed that impaired hae-
mostasis in the hearts of low TF mice leads to hemorrhage followed by inflammation and ultimately fibrosis. However, we cannot exclude the possibility that the TF:FVIIa complex may also contribute to signaling in cardiomyocytes and/or endothelial cells that influences cardiomyocyte survival and vessel stability. Cardiomyocytes and endothelial cells express both PAR-1 and PAR-2 (11, 35).

Role of TF in thrombosis

Aberrant TF expression initiates life-threatening thrombosis in a variety of diseases, including atherosclerosis, cancer and sepsis (4-6). For instance, rupture of atherosclerotic plaques exposes plasma clotting factors to high levels of TF with subsequent thrombosis (4). TF is also expressed by circulating monocytes and tumor cells that may initiate disseminated intravascular coagulation (5, 36). Finally, TF may be shed from cells into the circulation in the form of microparticles (37, 38). This circulating TF has been referred to as blood-borne TF and may contribute to thrombosis in different diseases.

Low TF mice had low levels of TF in the carotid artery (Day et al. submitted) and the whole blood clotting time was significantly prolonged using blood from low TF mice compared with wild-type mice (706 ± 145 and 341 ± 20, mean ± SE, n = 3). These data indicate that low TF mice have reduced TF in both the vessel wall and the blood. Low TF mice had prolonged occlusion times compared to wild-type mice in a carotid model of thrombosis involving oxidative damage to the vessel wall (Day et al. submitted). Similarly, in a heat-induced injury model of thrombosis using the microvasculature of the cremaster muscle, low TF mice had reduced TF and fibrin levels in the thrombus compared with wild-type mice (Chou et al. submitted). To determine the contribution of hematopoietic cell-derived microparticles to thrombosis in the two models, we employed bone marrow transplantation with either wild-type bone marrow or low TF bone marrow. We found that the occlusion time in the carotid thrombosis model was not affected by dramatically reducing TF in the hematopoietic cells (Day et al.
submitted). This result indicated that cells within the vessel walls were the primary source of TF-initiated clotting in this model. In contrast, reducing hematopoietic cell TF was associated with a significant reduction in TF and fibrin levels in the model of microvascular thrombosis (Chou et al. submitted). These data suggest that hematopoietic cells release TF positive microparticles into the circulation and that these microparticles contribute to thrombosis. Furie and colleagues have shown that microparticles bind to activated platelets within the thrombus via an interaction between PSGL-1 and P-selectin (39). Importantly, the two thrombosis models have several differences that may explain why hematopoietic cell-derived TF contributes to thrombosis after injury of the microvasculature but not after injury of the macrovasculature. Further studies are required to compare the role of blood-borne TF in thrombosis in different models, as well as in healthy and diseased mice where levels of blood-borne TF are likely to be increased.

Role of TF in pathologic and physiologic angiogenesis

Many studies suggest that TF plays an important role in pathologic angiogenesis. An early report by Nawroth and colleagues showed that overexpression of TF in Meth-A sarcoma cells implanted into mice enhanced vessel density and blood flow around the tumor (40). In addition, overexpression of TF was associated with increased expression of the proangiogenic molecule, VEGF, and decreased expression of the antiangiogenic molecule, thrombospondin-1 (40). Similarly, in human melanoma cells, TF expression correlates with VEGF expression (41). However, another study using human melanoma cells did not find a correlation between TF and VEGF expression (42). Clinical studies have shown a relationship between TF expression and VEGF expression, as well as a correlation between TF expression and microvessel density, which is an established marker for tumor angiogenesis (43-45).

Despite a plethora of studies, it is still unclear how TF contributes to angiogenesis. Tumor cells express high levels of TF and TF:FVIIa signaling may enhance angiogenesis via VEGF expression or by other mechanisms (46). TF expression by endothelial cells in the tumor vasculature is controversial. Rickles and colleagues reported TF expression by endothelial cells and found a correlation between TF expression and malignancy in human breast tumors (47). In contrast, Luther and colleagues failed to detect TF expression by endothelial cells in breast tumors (48). Further studies are required to identify the cell types that express TF during tumor angiogenesis, and to define the mechanism by which TF contributes to tumor angiogenesis. We believe that low TF mice provide a valuable tool to investigate the role of both host and tumor-derived TF in tumor angiogenesis.

Studies on the role of TF in physiologic angiogenesis are limited. In 1996, we and others showed that inactivation of the TF gene results in embryonic death at mid-gestation (E10.5) due to a defective yolk sac vasculature (23-25). It was suggested that TF may play both haemostatic and non-haemostatic roles in the maintenance of the yolk-sac vasculature. The non-haemostatic role of TF was proposed because 50% of PAR-1–/– embryos had a similar yolk sac vascular defect, whereas fibrinogen–/– embryos developed normally and were born at the expected rate (25, 49, 50). However, one can argue that platelets may provide sufficient haemostatic protection to allow embryonic development. More recently, it was shown that embryos lacking fibrinogen and thrombin-dependent activation of platelets (due to a deficiency in PAR-4) developed normally but died shortly after birth due to a severe haemostatic defect (51). The fact that 90% of TF null embryos die at mid-gestation, whereas embryos lacking both fibrinogen and PAR-4 do not, strongly argues that the death of TF null embryos is not simply due to a haemostatic defect. The most likely non-haemostatic role for TF in the maintenance of the yolk sac vasculature is in thrombin generation, which is required to activate PAR-1 on endothelial cells and to maintain vessel integrity (52).

We found that low levels of human TF rescued the yolk sac defect of murine TF null embryos (Fig. 6). Furthermore, a trans-

![Figure 7: Low TF mice have reduced TAT levels and increased survival in an endotoxemia model.](image-url)
gene expressing human TF lacking the cytoplasmic domain also rescued TF null embryos, whereas a transgene expressing a mutant human TF with reduced FVII/FVIIa binding did not (53). Similarly, embryos expressing murine TF lacking the cytoplasmic domain developed normally (54). These data suggest that the TF:FVIIa-thrombin-PAR-1 pathway plays a critical role in the maintenance of the yolk sac vasculature. Further studies are required to determine if TF contributes to physiologic angiogenesis in other vascular beds in adult mice. Currently, we are analyzing the role of TF in retinal angiogenesis using low TF mice.

### Role of TF in inflammation

During sepsis and endotoxemia, induction of TF within the vasculature causes systemic activation of the coagulation protease cascade (36). TF is expressed by circulating monocytes and possibly by microvascular endothelial cells (55-57). Inhibition of TF was shown to be protective in animal models of sepsis and endotoxemia by reducing both coagulation and inflammation (58-60). We found that low TF mice had reduced coagulation, inflammation and mortality compared to mTF+/−/hTF+ littermates in a model of endotoxemia (Fig. 7) (61). We analyzed the role of hematopoietic cell TF expression in endotoxemia by transplanting wild-type mice with bone marrow from either low TF mice or mTF+/−/hTF+ littermate controls. LPS-induced coagulation, inflammation and mortality were significantly reduced in wild-type mice receiving bone marrow from low TF mice compared to wild-type mice transplanted with bone marrow from control mice. However, endotoxemic wild-type mice transplanted with low TF bone marrow had higher plasma levels of TAT and IL-6 compared to those observed in endotoxemic low TF mice (61). This result suggests that both monocyte and endothelial cell TF may contribute to coagulation and inflammation during endotoxemia.

Based on in vitro studies (7-10), we hypothesized that cross-talk between coagulation and inflammation during endotoxemia was mediated by the activation of PAR-2 by the TF:FVIIa complex and/or the TF:FVIIa:FXa complex. However, PAR-2 deficiency did not affect inflammation or survival in a model of endotoxemia (61). Similarly, mice lacking PAR-1 expressed the same levels of IL-6 and had the same survival time as wild-type mice in the endotoxemia model. Importantly, combining thrombin inhibition with PAR-2 deficiency significantly reduced inflammation and increased survival compared with wild-type mice treated with hirudin (61). These results suggest that stimulation of both PAR-1 and PAR-2 by the coagulation proteases FVIIa, FXa and thrombin contributes to inflammation in endotoxemia.

### References


