Published in final edited form as:

Thromb Res. 2009 November ; 124(5): 516–520. doi:10.1016/j.thromres.2009.07.017.

Involvement of human decidual cell-expressed tissue factor in uterine hemostasis and abruption

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Abstract

Vascular injury increases access and binding of plasma-derived factor VII to perivascular cell membrane-bound tissue factor (TF). The resulting TF/VIIa complex promotes hemostasis by cleaving pro-thrombin to thrombin leading to the fibrin clot. In human pregnancy, decidual cell-expressed TF prevents decidual hemorrhage (abruption). During placentation, trophoblasts remodel decidual spiral arteries into high conductance vessels. Shallow trophoblast invasion impedes decidual vascular conversion, producing an inadequate uteroplacental blood flow that elicits abruption-related placental ischemia. Thrombin induces several biological effects via cell surface protease activated receptors. In first trimester human DCs thrombin increases synthesis of sFlt-1, which elicits placental ischemia by impeding angiogenesis-related decidual vascular remodeling. During pregnancy, the fibrillar collagen-rich amnion and choriondecidua extracellular matrix (ECM) provides greater than additive tensile strength and structural integrity. Thrombin acts as an autocrine/paracrine mediator that degrades these ECMs by augmenting decidual cell expression of: 1) matrix metalloproteinases and 2) interleukin-8, a key mediator of abruption-associated decidual infiltration of neutrophils, which express several ECM degrading proteases. Our recent observations that: 1) among the cell types at the maternal fetal interface at term TF expression is highest in decidual cells indicates that this TF meets the hemostatic demands of labor and delivery; 2) TF expression in cultured term decidual cells is enhanced by progesterin and thrombin suggest that maintenance of elevated circulating progesterone at term provides hemostatic protection, whereas abruption-generated thrombin can act in autocrine/paracrine fashion on DCs to promote hemostasis via enhanced TF expression.

INTRODUCTION

Tissue Factor (TF) regulates hemostasis

Tissue factor (TF) is a cell membrane-bound glycoprotein (MW 46 kDa) member of the class-2 cytokine receptor family. It is comprised of a hydrophilic extracellular domain, which acts as a receptor for factor VII or its active form, VIIa, a membrane-spanning hydrophobic domain, and a 21 amino acid cytoplasmic domain (1). Endothelial cells do not normally express TF;

Summary Sentence: During human pregnancy, thrombin formed by decidual cell-expressed tissue factor protects against decidual hemorrhage (abruption), but paradoxically promotes several abruption-related changes leading to preterm delivery.

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however TF is constitutively expressed at such perivascular sites as the adventitia of arteries and veins where it protects against hemorrhage following vascular injury (1,2). The TF/VIIa complex activates both Factor X and IXa to promote hemostasis via complex clotting factor-platelet interactions that ultimately convert prothrombin to thrombin, fostering hemostasis by fibrin clot formation (3). In mice and humans, TF expression conforms to a differential, tissue-specific pattern. In low TF expressing tissues like the liver, spleen, skeletal muscle and thymus, hemostasis depends primarily on the slower acting intrinsic coagulation pathway (Factor IXa). In high TF-expressing tissues like the placenta, dermis, heart, lung, brain and uterus, hemostasis is mediated primarily by the faster acting extrinsic coagulation pathway (Factor IXa). In the latter tissues, constitutive TF expression provides extra hemostatic protection against potentially fatal hemorrhage (1,4).

Transgenic TF mice establish the crucial role of TF during pregnancy

The absence of any genetic deficiency of TF in humans emphasizes its importance to species survival while obviating direct evaluation of the effects of TF deficiency in humans (5). Predictably, TF null mice experience early embryonic lethality by bleeding from fragile vessels (6). However, incorporation of a human minigene expressing TF at only 1% of wild type level rescued the knockout mice and produced live born pups. Adult low-TF mice display hemostatic defects in tissues that normally express high levels of TF (*i.e.*, heart, lung, uterus and decidua/placenta). In the mouse uterine wall, elevated TF expression by epithelial cells protects against hemorrhage during gestation as well as the post-partum period. By contrast, in low-TF expressing mice blood pools form in the placental labyrinth and there are high rates of fatal postpartum hemorrhage (5,6).

TF expression is tightly coupled to human decidualization during the menstrual cycle, gestation and in an *in vitro* model

Progesterone (P4) initiates decidualization (DZ) of estradiol (E₂)-primed human endometrial stromal cells during the mid-luteal phase of the menstrual cycle (7). Decidualizing stromal cells express TF mRNA and protein (8). Continued P4 stimulation induces the spread of TF-expressing decidualized stromal cells (decidual cells) throughout the late luteal phase and gestational endometrium (8). In cultured stromal cells isolated from pre-decidualized human endometrium (8)(9), medroxyprogesterone acetate (MPA) and P4 each enhance immunoreactive and functionally active TF levels. Although the stromal cells are unresponsive to E₂ alone, incubations with E₂ plus MPA elicits even greater up-regulation of TF levels (9). These differential ovarian steroid actions *in vitro* mimic E₂ priming of the endometrium for P4-induced DZ (9). Incubation with E₂ plus MPA, significantly enhances TF levels for at least three weeks (10). This prolonged up-regulation underscores the relevance of our *in vitro* DZ model for studying TF expression *in vivo*, since circulating E₂ and P4 levels as well as TF expressed by decidualized stromal cells are elevated during the luteal phase and across gestation. Chronic progestin-induced TF expression in cultured human endometrial stromal cells contrasts with transient up-regulation of TF expression induced by growth factors, cytokines and glucocorticoids in several other cell types in which TF mRNA levels are increased for only a few hours accompanied by an increase in TF protein levels lasting less than 24 hours (11). In other cell types, TF is transcriptionally regulated either by AP-1, or NF-kappa B, or early growth response factor-1 (Egr-1) or the Sp transcription family on the TF gene promoter (11). Transient transfections of human endometrial stromal cells with various TF promoter constructs determined that SP1 mediated basal as well as progestin-enhanced TF transcriptional activity (12,13). Immunostaining of human endometrial sections and cultured stromal cells demonstrated that progestin-regulated DZ *in vitro* and *in vivo* involves an increase in the ratio of Sp1 to its Sp3 antagonist (13).

Decidual TF protects against hemorrhage (abruption) throughout pregnancy

Implanting human blastocyst-derived syncytiotrophoblast invade the underlying stromal capillaries while extravillous trophoblast (EVT) subsequently breach spiral arteries enmeshed in a matrix of decidualized stromal cells. This process provides the developing embryo with a vital source of oxygen and nutrients and facilitates exchange of waste products prior to placentation (14). That it also risks local hemorrhage is evident from the occurrence of “chemical pregnancy” in which trophoblast derived human chorionic gonadotropin (HCG) is detected briefly in maternal blood followed by bleeding in the decidua (15). This threat of pregnancy-terminating bleeding is normally allayed by formation of a “hemostatic envelope” provided by P4-enhanced expression of TF in the surrounding DC (10). Human EVT continue to invade the decidua and penetrate the myometrium up to 20 weeks. Among species with a hemochorial placenta, human endometrium exhibits the most extensive DZ reaction and human EVT are the most intrinsically invasive (16).

Conversely, shallow EVT invasion results in incomplete decidual vascular conversion and inadequate utero-placental blood flow leading to placental ischemia and hypoxia. These events are well established in the pathogenesis of preeclampsia, intrauterine growth restriction and abruption (17,18). Placental lesions associated with abruption (decidual hemorrhage) include uteroplacental/spiral artery vascular thrombosis (19) and sclerotic lesions in myometrial arteries (20). The occurrence of decidual hemorrhage during and after the period of placentation is associated with spontaneous abortion, abruption and PTD (21). The extent of thrombin generation due to DC-expressed TF is indicated by the profound hypofibrinogenemia attendant severe abruption and the high sensitivity and specificity with which elevated circulating thrombin-antithrombin complex levels predict abruption-associated PTD [reviewed in (22)].

TF expression by decidual cells at term and preterm human placentas

In mice, TF expression by the uterine epithelium, decidua and trophoblast are reported to prevent fatal hemorrhage in the immediate postpartum period following detachment of the placenta from the uterine wall (4,5). In late human pregnancy, reports are conflicting as to the extent of TF expression by trophoblast [reviewed in (22)], whereas TF expression was not assessed in the decidua. In Fig. 1, placental specimens from idiopathic preterm and term deliveries were immunostained to localize both TF and the DC marker vimentin (Fig. 1B) in serial sections. In all specimen groups, TF expression in the decidua basalis was localized exclusively to the peri-membranous area of DCs and essentially absent from interstitial trophoblast. This contrast in differential TF localization is particularly evident where interstitial trophoblasts are immediately adjacent to and intercalate with DCs (Fig. 1A and 1C in idiopathic preterm and term tissues, respectively). In placental villi, TF immunostaining was present in the villous mesoderm localized primarily in peri-vascular adventitia (Fig. 1D, idiopathic preterm specimen). In contrast, syncytiotrophoblast and cytotrophoblast showed low TF staining. The HSCORE results (Fig. 1 legend) indicate that in both specimen groups TF immunostaining intensity was strongest in the peri-membranous area of DCs, and significantly higher than in interstitial trophoblasts and villous mesoderm ($P<0.05$). Immunostaining in the villous mesoderm exhibited moderate intensity, and was significantly higher than in interstitial trophoblasts ($P<0.05$). In summary, Fig. 1 indicates that the level of TF expression in DCs exceeds that of other cell types at the maternal-fetal interface. Localized at DC cell membranes this TF can readily bind to factor VII to meet the hemostatic demands of labor and delivery via thrombin formation.

Protease Activated Receptors (PARs) mediate cellular effects of thrombin and TF/VIIa

In addition to promoting hemostasis, thrombin induces several biological effects via cell surface PARs, a family of four distinct “seven membrane” G protein-coupled receptors. Each PAR ligand is a serine protease that binds to and cleaves its receptor to expose an N-terminal

tethered ligand (23) causing autoactivation. Thrombin induces proliferation and chemotaxis of pro-inflammatory immune and mesenchymal cells and activates endothelial cells via PAR 1, 3 and 4, whereas PAR-2 is primarily a receptor for trypsin and trypsin-like enzymes including the TF-VIIa complex (24,25).

Thrombin enhanced sFlt-1 expression in primary first trimester human DCs promotes local anti-angiogenesis

Soluble fms-like tyrosine kinase-1 (sFlt-1) (26) is a splice variant of the Flt-1 receptor for vascular endothelial cell growth factor (VEGF) that binds to and inactivates the angiogenic factors, VEGF and placental growth factor (PlGF). In cultured human leukocyte-free first trimester DCs, thrombin significantly enhanced steady-state mRNA and secreted protein levels of sFlt-1 while hirudin, a specific thrombin inactivator, blocked these effects (27). By contrast, neither tumor necrosis factor- α nor interleukin-1 (IL-1) β , affected sFlt-1 output. The specificity of thrombin-augmented sFlt-1 expression in first trimester DCs is further indicated by the absence of a thrombin effect on VEGF output as well as other placenta-expressed modulators of angiogenesis such as PlGF and endoglin (27). In contrast, thrombin did not affect sFlt-1 expression in cultures of DCs isolated from term placentas (27). These results suggest that excess thrombin-enhanced sFlt-1 expression in first trimester DCs could promote an anti-angiogenic milieu at the implantation site. That VEGF converts hematopoietic stem cells to endothelial cells (28) suggests that inhibition of local VEGF by DC-derived sFlt-1 impairs utero-placental blood flow by inhibiting EVT-mediated remodeling of spiral arteries and arterioles into low resistance, high capacity vessels. Since impaired vascular remodeling is linked to both pre-eclampsia and abruption, thrombin-augmented sFlt-1 output by first trimester DC-expressed TF could play an integral role in the pathogenesis of both conditions.

Thrombin effects on primary term human DCs promote placental separation and preterm premature rupture of the fetal membranes (PPROM) following abruption

Integration of bioengineering, histological and biochemical observations indicate that in human pregnancy the interactive effects of a fibrillar collagen-rich amnion and choriodecidua extracellular matrix (ECM) provide greater than additive tensile strength and structural integrity to withstand disruptive forces stemming from the fetus and myometrium (29). The weakest membrane component, the chorio-decidua, ruptures first followed by its amnion support structure (29). In abruption the superficial decidua basalis and placenta shears away from underlying decidua basalis. Targeting of collagens in the ECM of decidua basalis and choriodecidua/amnion, particularly collagens I and IV, by proteases released from neighboring cells impairs the strength of these ECMs (30). MMPs are the primary mediators of ECM degradation. The MMPs include: 1) collagenases, exemplified by interstitial collagenase (MMP-1), which efficiently degrade fibrillar collagens; 2) stromelysins, exemplified by stromelysin-1 (MMP-3), which degrade a broad array of ECM components and activates pro-MMP-1 and pro-MMP-9 to regulate an ECM-degrading cascade; and 3) gelatinases, exemplified by MMP-9 and MMP-2, which degrade collagen IV in the basement membrane component-enriched ECM (31). In cultured first trimester human DCs, thrombin markedly augments MMP-1, MMP-3 (32,33). Third trimester human decidua also contains abundant, constitutively expressed MMP-2 (34). A marked decidual infiltrate of neutrophils, which are a rich source of the ECM degrading proteases elastase and MMPs (35), are associated with abruption-induced PPRM in the absence of infection. Enhanced neutrophil trafficking into the decidua reflects thrombin-induced expression in DC of IL-8 (35), the primary neutrophil chemoattractant and activator (36,37). Thus, during abruptions thrombin-generated from decidual cell-expressed TF can promote decidual basalis shearing and PPRM directly via enhanced DC-expressed MMPs and indirectly by augmenting DC-expressed IL-8. The latter promotes trafficking into the decidua of neutrophils, which are rich in ECM-degrading proteases.

Thrombin-progestin interactions enhance TF expression in human term DCs

Figure 2 displays the separate and interactive effects of MPA and thrombin on immunoreactive levels of TF in cell lysates following incubation with leukocyte-free, term DCs. The choice of E₂ as the control incubation for E₂ plus MPA was predicated by elevated circulating E₂ and P4 levels during gestation. Compared with basal TF levels, thrombin and MPA each significantly enhanced TF levels by more than two-fold ($p < 0.05$). Thrombin added with E₂+MPA elicited at least an additive effect. The ELISA measurements in Fig. 2 were confirmed by Western blotting with the results of quantitative RT-PCR demonstrating corresponding changes in steady state TF mRNA levels (see (22) for details).

Summary

In human term and pre-term placental sections, immunostaining for TF in Fig. 1 indicates that levels of DC-expressed TF exceed that of other cell types at the maternal-fetal interface. This DC TF is localized at the cell membranes where it can bind to factor VII and meet hemostasis during parturition and following placental separation via thrombin formation. Previously, the only notable difference described for TF expression between human and mice was in the kidney with TF localized in the glomerulus in humans and primarily in the tubules in mice (38). However, Fig. 1 indicates that unlike elevated expression of TF in mouse trophoblast (4), but consistent with some reports in humans (2,39), third trimester human trophoblast express little or no TF. In contrast with the general concept that TF is constitutive in high expressing cells (1), Fig. 2 indicates that MPA and thrombin each significantly enhance TF expression in term DC monolayers and that incubation with MPA and thrombin elicit at least additive up-regulation of TF expression.

The crucial role played by P4 induced myometrial quiescence in maintaining pregnancy is well established in humans as well as many other mammalian species (40,41). However, Figs. 1 and 2 suggest that in humans, P4 is also critical in maintaining hemostasis towards the end of pregnancy and in the immediate post-partum period by maintaining elevated TF levels in DCs. This assertion is supported by the catastrophic hemorrhage that occurs when pregnancy complications accompany absent or impaired DZ as in ectopic pregnancy and placenta accreta. Thus, the absence of P4 withdrawal in humans avoids potentially fatal hemorrhage. Moreover, extrapolation of the *in vitro* results of Fig. 2 to the milieu at the maternal-fetal interface at term, suggests that abruption-generated thrombin acts in autocrine/paracrine fashion on DCs to up-regulate TF expression and thereby promote hemostasis.

Acknowledgments

This work was supported by grants from the National Institutes of Health: 2 RO1 HD 033937-12 (to C.J.L.) and the March of Dimes: 21-FY05-1249 (to C.J.L.).

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Figure 1. Immunohistochemical analysis of tissue factor (TF) expression at the decidual/placental interface

Serial sections of decidual basalis were immunostained for TF and vimentin in an idiopathic preterm specimen (A: TF, and B: vimentin). Decidual cells (DCs) (arrows), identified by positive vimentin staining, exhibited strong peri-membranous TF staining. TF staining was virtually absent in interstitial trophoblast (arrowheads). Similar results were seen in term specimens (C). Placental villi exhibited moderate TF staining in the mesoderm ("M"), where the staining was primarily localized to peri-vascular adventitia (small arrows), as shown in a preterm specimen (D). Syncytiotrophoblast ("S") and cytotrophoblast ("C") exhibited no TF immunostaining. Similar results were seen in term specimens (not shown). Negative control

immunostaining using a nonspecific isotype-matched antibody revealed no positive signals (E). A histogram of the HSCORE (mean $\pm$ SEM) of the intensity of TF immunostaining indicates highest staining in DCs (*, vs. interstitial trophoblast and the villous mesoderm of the same specimen group, $P < 0.05$), with moderate staining in the villous mesoderm ($\dagger$, vs. interstitial trophoblast in the same specimens, $P < 0.05$) (F) [see(22)].

Figure 2. Effects of MPA and thrombin on TF expression in decidual cells (DCs)

Confluent leukocyte-free term DCs, were primed in E_2 or E_2 + MPA then switched to a serum-free defined medium (DM) with corresponding steroids $\pm$ 2.5 U/ml thrombin (Th) for 24 hr. ELISAs were performed for TF in cell lysates with the results normalized to total cell protein: $n=10$ (mean $\pm$ SEM). * vs. E_2 ; ** vs. E_2 +MPA and E_2 + Th; $p<0.05$. [see (22)].