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Tissue factor is required for uterine hemostasis and maintenance of the placental labyrinth during gestation

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ABSTRACT **We employed a novel mouse line that expresses low levels of human tissue factor (TF) in the absence of murine TF to analyze the role of TF in gestation. Low-TF female mice had a 14–18% incidence of fatal postpartum uterine hemorrhage, suggesting that TF plays an important role in uterine hemostasis. Low-TF female mice mated with low-TF male mice had a 42% incidence of fatal midgestational hemorrhage ($n = 41$), whereas no fatal midgestational hemorrhages were observed in low-TF female mice mated with wild-type male mice ($n = 43$). Placentas of low-TF embryos from both low-TF and normal (+/−) TF females were abnormal and contained numerous maternal blood pools in the labyrinth. Placentas of TF null embryos surviving beyond embryonic day 10.5 exhibited similar defects. The mouse maternal–embryonic placental barrier consists of four cellular layers (layers I, II, and III and endothelial cells), where layer I lines the maternal lacunae. Comparison of TF-deficient placentas with control placentas by immunohistochemical and ultrastructural analyses revealed thinning of layer I and a reduction in the number of cellular contacts of layer I trophoblasts spanning the maternal blood space between adjacent trabeculae. These structural changes in low-TF and TF null placentas result in enlarged maternal lacunae, as determined by morphometric analysis, and placental hemorrhage, which leads to midgestational death of low-TF female mice. This study demonstrated that TF is required for uterine hemostasis and revealed an unexpected role of TF in the maintenance of the placental labyrinth.**

Tissue factor (TF) is a transmembrane glycoprotein and member of the class II cytokine receptor superfamily (1). TF functions as the major cellular initiator of blood coagulation (2, 3). Recent studies have suggested that TF plays a nonhemostatic role in the development and/or maintenance of blood vessels, cell signaling, and tumor metastasis (4–6). We and others reported that ≈90% of null embryos die at embryonic day (E)10.5 (7–9), which appears to be because of a defective yolk sac vasculature (7). Similarly, ≈50% of Factor V, prothrombin, or thrombin receptor (PAR-1) null embryos die at E10.5, which is a time when nutrition of the embryo depends on the yolk sac vasculature (10–14). Survival of all Factor VII null embryos beyond E10.5 (15) may be due to rescue by small amounts of maternal Factor VII. Finally, fibrinogen null mice are born at the expected frequency (16). These data suggest that the coagulation protease cascade plays an unexpected, non-fibrin-dependent role in embryonic yolk sac vasculature development. TF may contribute to the maintenance of the yolk sac vasculature at E9.5–E10.5, in part, by generating thrombin with subsequent activation of the cellular thrombin receptors.

TF also appears to play a role in the development and maintenance of tumor vasculature (5). Tumors in mice overexpressing TF produced more of the proangiogenic protein vascular endothelial cell growth factor (VEGF) and less of the antiangiogenic protein thrombospondin than controls (17), suggesting that TF regulates the balance of angiogenic factors. In humans, transient expression of TF on endothelial cells may enhance angiogenesis in malignant breast tumors (18). *In vitro* studies have shown that binding of Factor VIIa to TF increases the production of VEGF by fibroblasts (19). Taken together, these studies suggest that TF may contribute to the growth of the tumor vasculature by increasing VEGF expression.

Recently, we reported the rescue of mouse TF (mTF) null embryos with a human TF transgene (hTF) that expresses human TF from the human TF promoter (20). Adult "rescued" mice express low levels of human TF in a cell type-specific pattern that is similar to the pattern of mouse TF expression. Rescued mice ($mTF^{-/-}$, hTF^{+}) exhibited normal viability despite a low level of human TF activity (1% relative to mouse TF activity), suggesting that low levels of TF can maintain hemostasis compatible with life. In this study, we used low-TF mice to analyze the role of TF in gestation.

MATERIALS AND METHODS

Animals. All studies were approved by The Scripps Research Institute Animal Care and Use Committee and comply with National Institutes of Health guidelines. The generation of heterozygous TF mice ($mTF^{+/-}$), heterozygous TF mice carrying a human TF transgene ($mTF^{+/-}$, hTF^{+}), low-TF "rescued" mice ($mTF^{-/-}$, hTF^{+}), and TF null embryos ($mTF^{-/-}$) have been described (7, 20). Studies were performed by using two independent transgenic mouse lines (nos. 47 and 31) (20). C57BL/6J mice were obtained from The Scripps Research Institute animal core facility. Embryos (E13.5 and E15.5) from heterozygous and low-TF female mice were genotyped by Southern blotting (7, 20).

Immunohistochemical and Ultrastructural Analysis of Placentas. Embryos and placentas were either dissected free of the uterine wall or fixed *in situ* by using 4% paraformaldehyde in PBS. All subsequent histological analysis was performed by using paraffin-embedded tissue sections (3–4 μm). Sections were stained with hematoxylin and eosin for morphological analysis. Immunohistochemical analysis was performed after antigen retrieval as described previously (21). mTF and hTF in tissue sections from wild-type and low-TF mice were detected

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Abbreviations: TF, tissue factor; E, embryonic day; mTF and hTF, mouse and human TF, respectively.

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by using a sheep anti-rabbit TF polyclonal antibody (20 μg/ml) (22). Cytokeratin was detected by using a rabbit anticytokeratin antibody (1:1,000; Dakopatts, Glostrup, Denmark), cadherin was detected by using a rabbit anticadherin antibody (1:50; Sigma), and CD31 was detected with a rat anti-mouse CD31 mAb (10 μg/ml; PharMingen). Thereafter, sections were incubated with the corresponding peroxidase-labeled secondary antibodies or a biotinylated anti-rat antibody. Peroxidase activity was detected with diaminobenzidine (Aldrich). The biotinylated anti-rat antibody was detected with Vectastain Elite ABC linked to alkaline phosphatase, and enzyme activity was detected with Vector red (Vector Laboratories). Controls included omissions of the primary antibody or replacement by nonimmune IgG (data not shown).

Placentas were collected for electron microscopy after perfusion of mice with PBS followed by PBS containing 2% glutaraldehyde and 4% paraformaldehyde. Placentas were isolated as above, fixed overnight in the perfusate, washed in 0.1 M cacodylate buffer, fixed with osmium, and embedded in resin. Thick sections were cut and stained with toludine blue for orientation, and ultra-thin sections were stained with uranyl acetate and lead citrate for assessment by electron microscopy (GM100; Philips, Eindhoven, the Netherlands).

***In Situ* Hybridization.** Expression of mouse TF mRNA in wild-type placentas was examined by *in situ* hybridization by using ^{35}S-labeled RNA riboprobes (23).

Quantitation of Placental TF mRNA and Functional Activity. Quantitative PCR was used to determine the levels of mouse and human TF mRNA from placentas of wild-type and low-TF embryos, respectively (20). TF activity in placental extracts from wild-type and low-TF embryos was determined by using a one-stage clotting assay with mouse plasma (Sigma) (20). TF activity was calculated in arbitrary units by reference to a standard curve for purified human TF. Human TF activity was inhibited by preincubating placental extracts with inhibitory anti-human TF mAbs (TF8–5G9, TF8–6B4, and TF9–9C3) (24).

Morphometric Analysis of the Size of Maternal Lacunae. The size of maternal lacunae in placental sections (E15.5) from normal (+/−) TF ($n = 4$), low-TF ($n = 3$), and TF null ($n = 1$) placentas was determined with a light microscope with drawing tube (Zeiss) by using a digitizer tablet (Kontron, Zurich). The mean profile area of lacunae was measured in five optical fields from each placenta (total area analyzed = 0.0622 μm^2).

Statistical Analysis. Values for quantitative PCR and clotting were reported as the mean ± SD, and statistical significance was determined by the unpaired Student's *t* test. The size of maternal lacunae was expressed as mean area ± SEM, and statistical significance was determined by Mann–Whitney *U* test.

RESULTS

Expression of Mouse and Human TF in the Uterus and Placenta. We have generated a novel mouse line in which expression of human TF from a transgene rescues the embryonic lethality of mTF$^{-/-}$ embryos (20). We employed this "rescued" mouse line to determine the role of TF in gestation. Quantitation of TF mRNA expression indicated that the level of human TF mRNA ($6.4 \pm 4.9 \times 10^6$ molecules/ng RNA, $n = 3$) in placentas of low-TF embryos (E13.5) from low-TF female mice was 0.5% of wild-type levels of mouse TF mRNA ($1.3 \pm 0.75 \times 10^9$ molecules/ng RNA, $n = 3$) ($P < 0.02$). Similarly, human TF activity in placentas from low-TF embryos (57 ± 10 units/mg protein, mean ± SD, $n = 3$) was 0.7% of mouse TF activity in wild-type placentas (7,998 ± 2,286 units/mg protein, $n = 3$) ($P < 0.01$). *In situ* hybridization experiments demonstrated that mouse TF mRNA was expressed strongly in uterine epithelial cells and expressed weakly in trophoblasts within the placental labyrinth (Fig. 1 *A–C*). The level of human TF mRNA expression was below the detectable threshold of the *in situ* hybridization experiments. Immunohistochemical analysis showed that mouse and human TF proteins were expressed in uterine epithelial cells of wild-type mice and low-TF mice, respectively (Fig. 1 *D–G*). These results indicated that low levels of human TF mRNA were expressed in placentas of low-TF embryos and that the cell type-specific pattern of human TF protein expression in mouse uterine epithelium of low-TF mice was similar to the pattern of mouse TF expression in wild-type mice.

Fatal Postpartum Uterine Hemorrhage in Low-TF Mice. We established various breeding strategies to examine the role of TF in postpartum hemostasis (Table 1). In mouse parturition, after delivery of the pups, placentas separate from the uterine wall and are delivered vaginally. Fatal postpartum uterine hemorrhage was observed in low-TF female mice bred with low-TF male mice (18%) and in low-TF female mice bred with wild-type male mice (14%) (Table 1). Fatal uterine hemorrhage occurred within 24 h of parturition. No fatal postpartum uterine hemorrhages were observed in heterozygous TF females bred with low-TF male mice (0/273) (Table 1), which gave birth to low-TF and heterozygous TF pups. Heterozygous TF mice contain levels of TF that are functionally indistinguishable from wild-type mice (data not shown) and will be referred to as normal (+/−) TF mice. These data demonstrated that low-TF female mice exhibited fatal postpartum hemorrhage independent of the genotype of the pups.

Fatal Midgestational Hemorrhage in Low-TF Mice. Breeding of low-TF female mice with low-TF male mice resulted in a fatal midgestational hemorrhage in 42% (17/41) of the pregnancies between E12 and E16 (Table 1). In contrast, no fatal midgestational hemorrhages were observed in low-TF

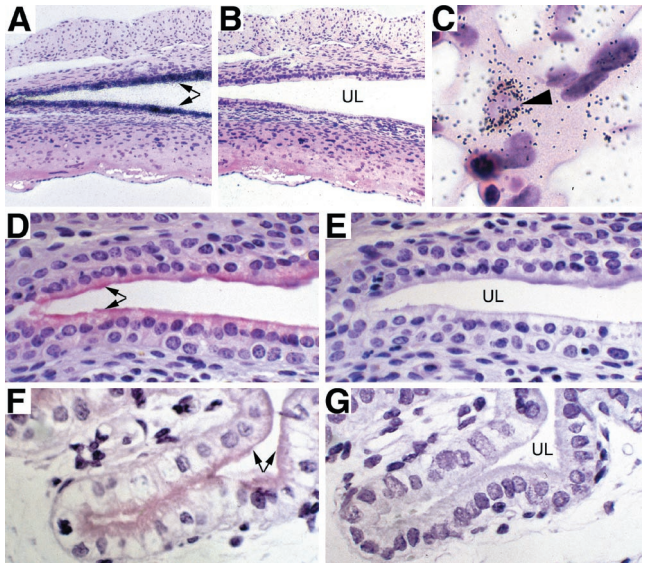

FIG. 1. TF expression in the uterus and placenta. *In situ* hybridization experiments were performed to determine mouse TF mRNA expression in the uterus and placenta (E13.5) of a wild-type mouse (*A–C*). Uterine sections from a wild-type mouse were incubated with antisense (*A*) or sense (*B*) mouse TF riboprobes (×100). (*C*) The labyrinthine zone of a wild-type placenta incubated with a mouse TF antisense riboprobe (×1,000). Arrowhead indicates a mouse TF mRNA-positive trophoblast within the placental labyrinth. Immunohistochemical analysis was used to localize mouse and human TF in the uterus of wild-type and low-TF mice, respectively. Uterine sections from a wild-type mouse were incubated with a sheep anti-rabbit TF polyclonal antibody (*D*) or a control antibody (*E*) (×400). Uterine sections from a low-TF mouse were incubated with a sheep anti-rabbit TF polyclonal antibody (*F*) or a control antibody (*G*) (×400). Arrows indicate TF-positive uterine epithelium. UL, uterine lumen.

Table 1. Fatal hemorrhage in low-TF female mice

Breeding strategy	Level of TF expression		Fatal mid-gestational hemorrhage (E12–E16)	Fatal post-partum hemorrhage	Placental blood pools
	Mother	Embryo			
♂ $-/-$, h^+ × ♀ $-/-$, h^+	Low	Low	17/41 (42%)	4/22 (18%)	Yes
♂ $+/+$ × ♀ $-/-$, h^+	Low	Normal (+/−)	0/43	6/43 (14%)	No
♂ $-/-$, h^+ × ♀ $+/-$, h^+	Normal (+/−)	Normal (+/−)	0/273	0/273	No
	Normal (+/−)	Low			Yes

The breedings in the first row employed 6 male and 25 female mice. The breedings in the second row employed 5 male and 10 female mice. h^+ represents $h^{+/-}$ or $h^{+/+}$.

female mice bred with wild-type male mice (0/43) (Table 1). These results demonstrated that a low level of embryonically derived TF in the placenta led to the death of many low-TF female mice. To determine whether the presence of a low-TF embryo increases the risk of midgestational hemorrhage in normal (+/−) TF female mice, normal (+/−) TF female mice were mated with low-TF male mice. In this breeding, half of the embryos and placentas will contain low levels of human TF with no mouse TF and half will contain functionally normal (+/−) levels of mouse TF. No fatal midgestational hemorrhages (0/273) were observed in normal (+/−) TF mice carrying low-TF and normal (+/−) TF embryos (Table 1). All breedings contained a combination of $h^{+/-}$ (66%) and $h^{+/+}$ (33%) mice. Taken together with the high mortality of TF null embryos at E10.5 (≈90%), we expect a very low frequency of TF null embryos without the hTF minigene in these breedings at E12–E16. In addition, no TF null embryos (E12–E16, n = 98) were observed in sacrificed $mTF^{+/-}$, h^+ female mice crossed with $mTF^{-/-}$, h^+ male mice, suggesting that TF null embryos did not contribute significantly to the fatal midgestational death of low-TF female mice. These data indicated that fatal midgestational hemorrhage in low-TF female mice required both low levels of embryonically derived TF in the placenta and low levels of maternal TF in the decidua and uterus.

Defects in the Placental Labyrinth of Low-TF Embryos. To determine whether placentas of low-TF embryos were abnormal, we analyzed placentas of low-TF embryos from both low-TF and normal (+/−) TF female mice (Table 1). Macroscopic examination revealed numerous blood pools within almost all placentas of low-TF embryos from both low-TF and normal (+/−) TF female mice, whereas no blood pools were observed in placentas of age-matched and normal-littermate (+/−) TF embryos (Table 1) (Fig. 2). No abnormalities were observed in the yolk sac vasculature of low-TF embryos at E9.5 (data not shown) or E13.5 (Fig. 2*A*), and no blood pooling was observed within the yolk sac, consistent with the observed 100% survival of low-TF embryos carried by normal (+/−) TF female mice. These results indicated that reducing the level of embryonically derived TF is associated with placental defects that are independent of the maternal genotype.

Microscopic analysis of the blood pools within low-TF placentas at E13.5 revealed that they were present in the placental labyrinth beneath the chorioallantoic plate (Fig. 3 *A–C*). Careful inspection of the blood pools revealed that they consisted of maternal red blood cells within enlarged maternal lacunae and did not contain embryonic nucleated red blood cells. Intraplacental hemorrhages were observed in placentas of older (E15.5) low-TF embryos but not in normal (+/−) littermate controls (Fig. 3 *D* and *E*). Placentas of low-TF embryos (E12–E16; see Table 1) from low-TF female mice (n = 17) that died of midgestational hemorrhage exhibited ruptured placental blood pools, particularly at the shoulder region of the labyrinthine layer (data not shown). In addition, we observed maternal blood pools in placentas of low-TF embryos derived from an independent founder line (no. 31) containing the human TF transgene and no mouse TF (data not shown). Maternal blood pools and placental hemorrhages also were observed in four of five placentas of E13.5 TF null embryos (not shown) and two of two placentas of E15.5 TF null embryos but not in normal (+/−) TF littermate controls (Fig. 3 *F* and *G*). These data indicated that a reduction or absence of embryonically derived TF in the placenta results in placental defects within the labyrinthine layer.

The mouse placental labyrinth is composed of an intricate network of maternal blood spaces and embryonal blood vessels. Maternal and embryonal blood is separated by a placental barrier that consists of four cellular layers: layer I trophoblasts line the maternal lacunae, trophoblasts in layers II and III form syncytial-like layers, and endothelial cells line the embryonic vessels (25) (see Fig. 7). Immunohistochemical analysis of the placental labyrinth of normal (+/−), TF null, and low-TF placentas was performed to determine whether there were structural abnormalities in the placental barrier of TF-deficient placentas. An epithelial cell lineage-specific antibody to cytokeratin, an endothelial cell lineage-specific antibody to CD31, and an antibody to cadherin were used to identify the cellular components of the placental barrier. No differences in cellular distribution of both cadherin and CD31 staining were observed between TF null, low-TF, and normal (+/−) TF placentas (Fig. 4 *A* and *B* and data not shown). However, comparison of the pattern of cytokeratin staining revealed reduced staining of layer I in TF null and low-TF placentas

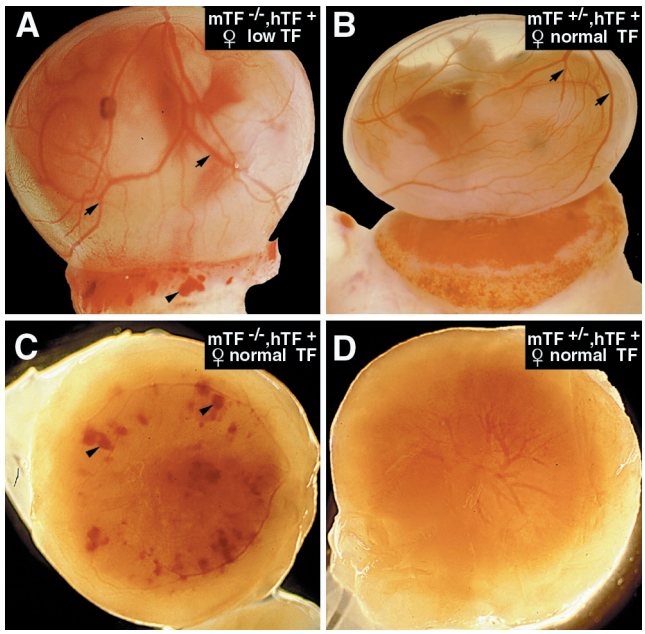

FIG. 2. Macroscopic analysis of low-TF placentas. Macroscopic views of low-TF (*A*) and normal (+/−) TF (*B*) placentas and embryos in their yolk sacs at E13.5 from low-TF and normal (+/−) TF female mice, respectively. Placentas of low-TF (*C*) and normal (+/−) TF (*D*) littermates at E13.5 from normal (+/−) TF female mice viewed from the embryonic surface (embryo removed). Arrows in *A* and *B* indicate yolk sac blood vessels. Arrowheads in *A* and *C* show blood pools within the labyrinthine layer of the placenta.

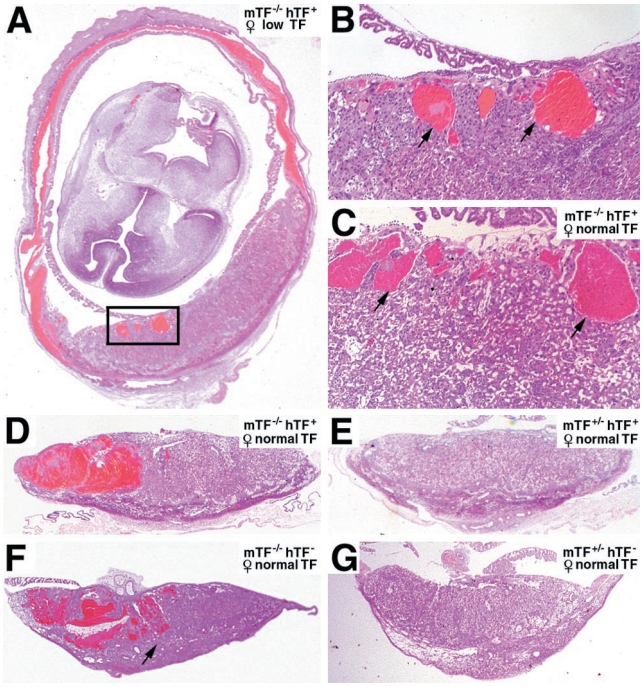

FIG. 3. Placental defects in low-TF and TF null placentas. Transverse sections of a low-TF embryo and placenta (E13.5) from a low-TF female (whole mount). (*B*) Higher magnification (×40) of blood pools in the low-TF placenta from *A*. (*C*) Blood pools in a placenta of a low-TF embryo (E13.5) from a normal (+/−) TF female (×40). Transverse sections of placentas of low-TF (*D*) and normal (+/−) TF (*E*) littermate embryos (E15.5) from a normal (+/−) TF female. Transverse sections of placentas of TF null (*F*) and normal (+/−) TF (*G*) littermate embryos (E15.5) from a heterozygous TF female. Arrows in *B*, *C*, and *F* indicate maternal blood pools within the placental labyrinth.

compared with placentas of normal (+/−) TF littermates at stage E15.5 (Fig. 4 *C* and *D* and data not shown).

In normal (+/−) TF and wild-type placentas, cellular processes from layer I trophoblasts formed cell–cell contacts that spanned the maternal blood space between adjacent trabeculae leading to a subdivision of maternal lacunae. Analysis of TF null placentas revealed significantly less cellular contacts between trophoblast I cells and adjacent trabeculae (Fig. 4). Morphometric analysis revealed that the mean profile area of maternal lacunae was significantly ($P < 0.05$) larger in both low-TF (799 ± 123 μm^2, mean ± SEM) and TF null (987 ± 103 μm^2) placentas compared with those present in normal (+/−) TF (387 ± 51 μm^2) placentas. Ultrastructural analysis of the placental barrier showed a reduction in the number of cellular contacts between layer I trophoblasts that subdivide maternal lacunae in low-TF placentas compared with those present in wild-type placentas. The cellular contacts that remained in low-TF placentas had reduced areas of cell–cell contact compared with their wild-type counterparts (Fig. 5 *A–D*). In addition, we observed thinning of layer I at E13.5 (data not shown) and at E15.5 (Fig. 5 *E* and *F*) in low-TF placentas compared with control placentas. Examination of the placental barrier surrounding a maternal blood pool revealed a focal loss of layer I (Fig. 5 *H* and *I*). Taken together, these results suggested that a reduction or absence of TF in the placenta leads to (*i*) structural abnormalities in trophoblast layer I of the labyrinthine zone, (*ii*) enlarged size of maternal lacunae, and (*iii*) formation of maternal blood pools and subsequent placental hemorrhage.

DISCUSSION

Low levels of human TF expressed from a transgene are sufficient to rescue the embryonic lethality observed in mTF

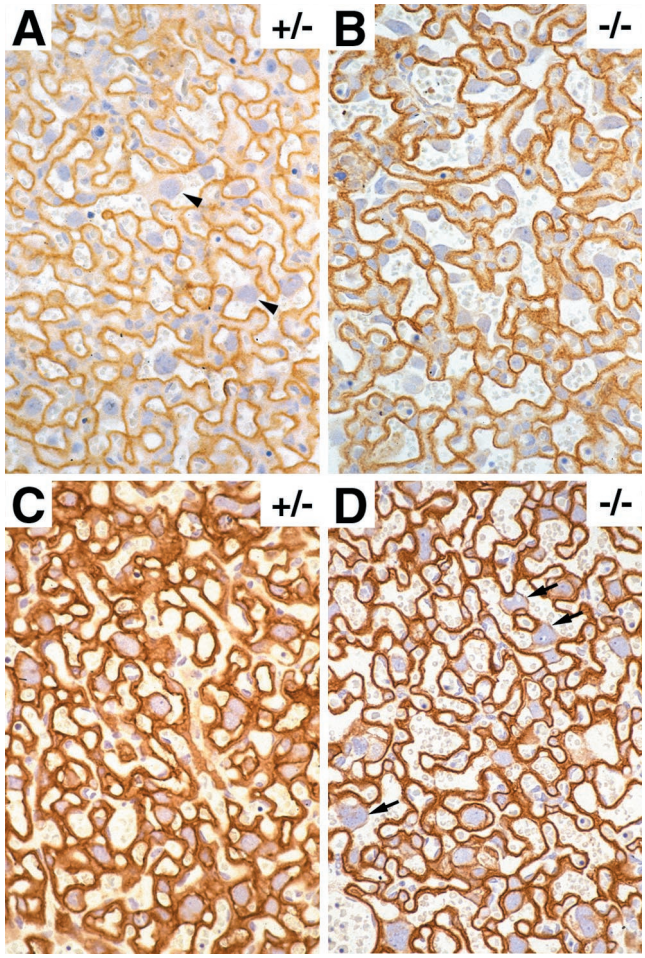

FIG. 4. Immunohistochemical analysis of the placental labyrinth in TF null and normal TF (+/−) placentas. Placental sections from normal TF (*A* and *C*) and TF null (*B* and *D*) embryos were incubated with an anticadherin antibody (*A* and *B*) or an anticytokeratin antibody (*C* and *D*). Arrowheads identify layer I trophoblasts with multiple cellular contacts to adjacent trabeculae in normal (+/−) placentas (*A*). Note the larger maternal lacunae and reduced cytokeratin staining in layer I trophoblasts of TF null placentas (arrows in *D*) compared with the placentas from normal (+/−) TF placentas. (×400.)

null mice (20). Adult low-hTF mice are fertile and have normal hemostasis after tail biopsy (20). In this study, we report fatal postpartum uterine hemorrhage in low-TF female mice. Human TF protein was expressed in the uterine epithelium of rescued mice, indicating that postpartum hemorrhage is not simply due to a lack of cell type-specific human TF expression. A similar number of fatal postpartum hemorrhages were observed with low-TF female mice bred with low-TF male mice (18%) and wild-type mice (14%), indicating that hemorrhage was a result of reduced TF expression in the female mice and was independent of the genotype of the pup. These results demonstrate that expression of normal levels of TF in the uterine epithelium and decidua are required to prevent fatal hemorrhage in the immediate postpartum period.

Fatal midgestational hemorrhage was observed in low-TF female mice bred with low-TF male mice (17/41) but not in those bred with wild-type male mice (0/43), which indicated that a low level of embryonically derived TF expression in the placenta led to death of low-TF female mice with impaired uterine hemostasis. In contrast, we presume that the lack of fatal hemorrhages in normal (+/−) TF female mice carrying low-TF embryos with placental hemorrhages (0/273) is due to adequate decidual and uterine hemostasis of heterozygous TF

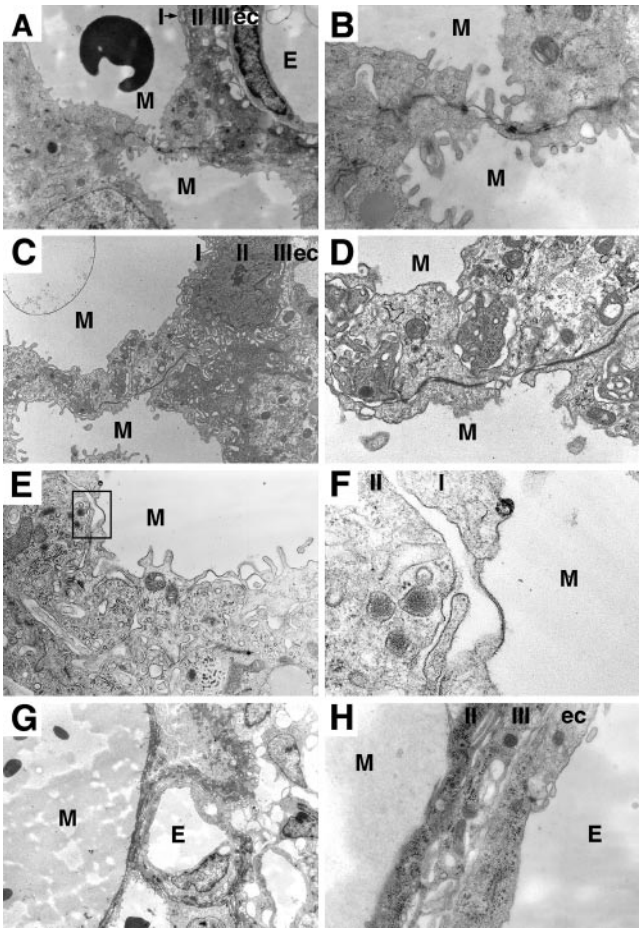

FIG. 5. Ultrastructural analysis of the placental barrier in low-TF placentas. The areas of cellular contact between layer I trophoblasts that subdivide maternal lacunae were reduced in low-TF placentas [×5,200 (*A*); ×15,500 (*B*)] compared with similar cellular contacts in wild-type placentas [×5,200 (*C*); ×15,500 (*D*)] at E15.5. Low-TF placentas (E15.5) exhibited thinning of layer I [×12,500 (*E*); ×25,000 (*F*)]. Layer I was absent in the placental barrier surrounding a maternal blood pool in a low-TF placenta (E15.5) [×2,200 (*G*); ×15,500 (*H*)]. M, maternal lacunae; E, embryonic vessel. The maternofetal barrier layers are indicated in *A*, *C*, *F*, and *H* (ec, embryonic endothelial cell; trophoblast layers I, II, and III).

mice. Fibrinogen null female mice exhibit uniform fatal midgestational hemorrhage at E9–E10 of development, which may be because of a failure of hemostasis during embryonic trophoblast invasion of maternal vessels (16). In contrast to our results with low-TF mice, fatal hemorrhage in fibrinogen null mice was independent of the embryonic genotype. Surprisingly, Factor IX null female mice give birth without hemorrhagic complications (26). Taken together, these results demonstrate that the extrinsic pathway of blood coagulation is required for uterine hemostasis during gestation and in the puerperium.

Early development and organization of definitive placentas (E9.5) in both low-TF and TF null embryos appeared normal (8) (data not shown). Maternal blood pools and placental hemorrhage were observed in the labyrinthine layer between E12 and E16 in placentas of low-TF embryos from both low-TF and normal (+/−) TF female mice. Maternal blood pools also were observed in placentas of TF null embryos (E13.5 and E15.5) surviving beyond E10.5 and in low-TF placentas from an independent founder (no. 31). These results indicated that a reduction or absence of embryonically derived TF in the placenta is associated with defects in the labyrinthine zone at a time when the placenta matures and the labyrinthine zone increases in volume (25). Normally, rapidly growing trophoblasts of layer I form a continuous lining for the maternal blood space during this gestation period. A study using a golden hamster has shown attenuation of this layer at later stages of gestation (25). In low-TF and TF null placentas we observed by immunohistochemical and ultrastructural analysis a premature thinning of layer I and a reduction in the number of cellular contacts to adjacent trabeculae compared with control placentas. These structural changes result in progressive enlargement and coalescence of maternal lacunae and subsequent placental hemorrhage. Early placental hemorrhage (E9.5–E10.5) has been reported in embryos null for a variety of proteins, including transcription factors, growth factors, and integrins (27–29). Later placental hemorrhage (E13.5–E15.5) has been observed in placentas lacking the leukemia inhibitory factor receptor (30). These placentas contained blood-filled spaces in the labyrinthine layer in a manner similar to TF-deficient placentas. Interestingly, lymphocyte inhibitory factor has been demonstrated to temporally regulate trophoblast protease generation (31).

At present, it is not known how TF contributes to the maintenance of the labyrinthine zone of the placenta. We cannot formally exclude the possibility that reduced TF expression in the placenta results in impaired hemostasis and focal hemorrhage that affects the structural integrity of layer I. However, on balance a hemostatic disturbance seems unlikely because no defects have been reported in placentas of Factor VII, Factor V, prothrombin, or fibrinogen null embryos (10, 12, 13, 15, 16). Importantly, expression of low levels of human TF rescues the vascular integrity defect in the yolk sac of murine TF null embryos at E9.5–10.5 but fails to rescue the midgestational defects in the definitive placenta, suggesting that TF has additional roles in the maintenance of the placenta. The function of these maternofetal transport systems depends not only on biochemical regulation of transfer within the membrane but also on the structure of the transporting barriers. Our study demonstrates that a reduction or absence of TF in the placenta results in a structural abnormality in layer

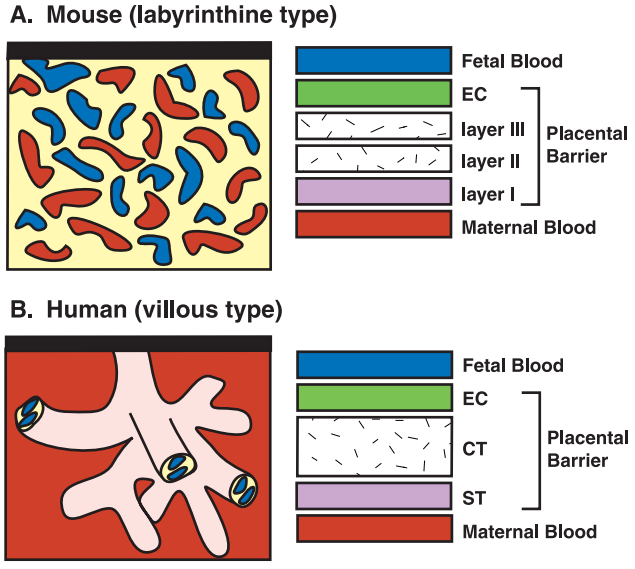

FIG. 6. Structure of the maternofetal barrier in mouse and human placentas. The mouse and human placentas have labyrinthine and villous types of interdigitation between maternal and fetal tissues, respectively. Maternal blood is red, fetal blood is blue, and fetal trophoblasts are black. Mice and humans form hemotrichorial placentas. In mice, the maternofetal barrier consists of four layers: (*i*) a fetal endothelial cell layer (EC), (*ii*) trophoblast layer III, (*iii*) trophoblast layer II, and (*iv*) trophoblast layer I. In humans, three layers are present: (*i*) a fetal endothelial cell layer (EC), (*ii*) a cytotrophoblast layer (CT), and (*iii*) a syncytialtrophoblast layer (ST).

I. Although the exact pathogenetic mechanism that causes this placental defect is still not clear, TF may play a nonhemostatic role in placental maintenance by affecting the regulation of protease generation, by initiating Factor VII-dependent cell signaling or by a direct role in cellular adhesion (32–35). Further studies are required to elucidate how TF contributes to the maintenance of the placental labyrinth.

In conclusion, this study demonstrates an important role for TF in postpartum uterine hemostasis in a mouse model. This observation is consistent with the fact that women with congenital deficiencies in Factor VII, fibrinogen, and Factor XIII exhibit severe postpartum hemorrhage (36–38). There have been no reports of congenital deficiencies in TF, but we speculate that reduced TF expression in the uterine epithelium may be associated with hemorrhagic complications in humans during childbirth. The placentas of mice and humans contain essentially the same basic maternal and fetal tissues and perform the same functions but exhibit important structural differences (Fig. 6). It is therefore difficult to draw exact parallels between the morphological alterations in TF-deficient mouse placentas and aberrant conditions of human pregnancy. Nevertheless, aberrant TF expression may underlie some unexplained placental hemorrhages observed in humans during gestation.

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