Fetal-Derived Trophoblast Use the Apoptotic Cytokine Tumor Necrosis Factor-α–Related Apoptosis-Inducing Ligand to Induce Smooth Muscle Cell Death

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Abstract—Remodeling of the uterine spiral arteries during pregnancy transforms them from high to low resistance vessels that lack vasoconstrictive properties. This process is essential to meet the demand for increased blood flow imposed by the growing fetus. Loss of endothelial and smooth muscle cells (SMC) is evident in remodeled arteries but the mechanisms underlying this transformation remain unknown. This study investigated the hypothesis that fetal trophoblast invading from the placenta instigate remodeling by triggering cell death in vascular SMC. Specifically, a role for trophoblast-derived death inducing cytokine tumor necrosis factor-α–related apoptosis-inducing ligand (TRAIL) was investigated. Expression of the activating TRAIL receptors R1 and R2 was detected by flow cytometry on human aortic SMC and by immunohistochemistry on spiral artery SMC. Recombinant human TRAIL induced human aortic SMC apoptosis, which was inhibited by antibodies against TRAIL-R1 or -R2. Perfusion of denuded spiral artery segments with recombinant human TRAIL also induced SMC apoptosis. Trophoblasts isolated from first trimester placenta expressed membrane-associated TRAIL and induced apoptosis of human aortic SMC; apoptosis was significantly inhibited by a recombinant human TRAIL-R1:Fc construct. Trophoblast within the first trimester placental bed also expressed TRAIL. These data show that: 1) TRAIL causes SMC death; 2) trophoblast produce the apoptotic cytokine TRAIL; and 3) trophoblast induce SMC apoptosis via a TRAIL-dependent mechanism. We conclude that TRAIL produced by trophoblast causes apoptosis of SMC and thus may contribute to SMC loss during spiral artery remodeling in pregnancy. (***Circ Res*. 2007;100:834-841.**)

Key Words: apoptosis ■ cell death ■ pregnancy ■ vascular remodeling ■ vascular smooth muscle

Remodeling of the uterine spiral arteries commences during the first trimester of human pregnancy and is completed by around 20 weeks of gestation. During this time, vessels lose their vaso-responsiveness because of loss of endothelial and smooth muscle cells (SMC), which are replaced with fetal-derived trophoblast in a fibrinoid matrix. This process, termed physiological change, results in transformation of the vessels from narrow to large calibre conduits and is essential to enable the oxygen and nutrient demands of the growing fetus to be met.[1] Histological evidence suggests a role for trophoblast in this process, as shallow trophoblast invasion and incomplete remodeling have been associated with fetal growth restriction,[2] early onset of labor[3] and pre-eclampsia.[4,5]

The mechanisms underlying spiral artery remodeling are still yet to be elucidated, largely because of the difficulty in obtaining tissue and the scarcity of suitable animal models that mirror the highly invasive nature of human hemochorial placentation.[1,6] Much of the current understanding is derived from examination of histological specimens and studies of primate models. Trophoblast, derived from chorionic villi, migrate away from the placental villous tips forming a population termed extravillous trophoblast. These cells form two populations, defined by the route with which they invade the surrounding tissue. Interstitial trophoblast invade the decidual stroma, migrate across the endometrial-myometrial boundary and progress into the first third of the myometrium. Endovascular trophoblast invade via the lumens of spiral arteries, however the source of these cells is unclear. Although it is possible that they may enter the vessel lumen directly and migrate against the flow of blood, it is more likely that they arise from an interstitial population that intravasate into the artery lumen.[7,8] By adopting a vascular-like phenotype extravillous trophoblast are able to infiltrate the spiral arteries and interact with cells of the vessel walls.[9] We have shown that endothelial cells and smooth muscle

Original received January 11, 2006; first resubmission received August 21, 2006; second resubmission received January 19, 2007; accepted February 12, 2007.

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Circulation Research **is available at http://circres.ahajournals.org** **DOI: 10.1161/01.RES.0000261352.81736.37**

cells are lost from spiral arteries by trophoblast-induced apoptosis through a mechanism involving activation of the Fas/Fas ligand (FasL) pathway.[10,11] The aim of this study was to investigate other apoptotic ligands produced by trophoblast that may be involved in causing apoptosis of SMC in the vessel wall.

Tumor necrosis factor (TNF)-α–related, apoptosis-inducing ligand (TRAIL) is a member of the TNF family of death-promoting ligands. It is expressed on the surface of cells in a membrane bound form, the carboxy-terminus of which can be cleaved by cysteine proteases generating a soluble form.[12,13] TRAIL is known to be produced by the placenta, and mRNA for TRAIL was upregulated in two trophoblast cell lines following cytokine stimulation.[14–16] TRAIL transduces its apoptotic signal via receptor trimerization and activation of the extrinsic pathway of apoptosis. The adaptor molecule Fas-associated death domain (FADD) binds to the intracellular death domain (DD) of the clustered receptors and recruits and activates caspase-8, which initiates activation of the caspase cascade leading to apoptosis (reviewed in[13,17,18]).

TRAIL signaling is made more complex by the existence of five different receptors: TRAIL-receptor 1 (TRAIL-R1) and TRAIL-receptor 2 (TRAIL-R2) contain intracellular DDs and can signal to cause apoptosis; TRAIL-receptor 3 (TRAIL-R3) lacks a DD, and TRAIL-receptor 4 (TRAIL-R4) has a truncated DD, neither of which can signal and are thought to act as decoy receptors; and finally a fifth, soluble receptor, osteoprotegerin, can bind and sequester TRAIL, although its importance in regulating TRAIL signaling is yet to be clearly defined.[13,19] Despite the initial theory that the relative expression of activating and decoy receptors may determine a cell's susceptibility to TRAIL-induced apoptosis, no such link has been definitively demonstrated.[18]

Much interest in TRAIL has arisen from the observation that TRAIL appears to selectively induce apoptosis in tumor or transformed cells, with no apparent effect on normal cells. This led to many studies examining the potential of using TRAIL in cancer treatment, exploiting two sought after attributes of selectivity and low toxicity compared with other pro-apoptotic cytokines such as FasL or TNF-α.[17,20,21] However, TRAIL has been found to induce apoptosis in normal human hepatocytes[22] and normal human prostate epithelial cells,[23] and its effects appear not only cell but species specific.[19,21] Indeed, many normal human cells express the activating receptors TRAIL-R1 and TRAIL-R2,[24,25] suggesting TRAIL may have physiological roles under normal conditions.

The effects of TRAIL on vascular cells have not been extensively investigated. Both endothelial[26] and SMC[27] have been shown to express TRAIL receptors, and survival and proliferation of both cell types has been reported to be stimulated by TRAIL.[27,28] In direct contrast, TRAIL has also been found to induce apoptosis of endothelial cells[26] and SMC.[29] A recent article has demonstrated that vascular SMC in atherosclerotic plaques are killed by TRAIL-expressing T cells.[30] Clear delineation of the impact of TRAIL on the vasculature is thus warranted, particularly given this recent interest in its role in atherosclerotic plaque stability.

This study examines the hypothesis that vascular SMC apoptosis plays a key step in remodeling uterine spiral arteries during human pregnancy. Specifically, we focused on the interaction between trophoblast and SMC, and the potential involvement of TRAIL in trophoblast-induced SMC apoptosis. Our results define a physiological role for TRAIL during pregnancy and identify a mechanism that contributes to SMC loss during vessel remodeling. This finding has major implications for both the mechanism of vessel remodeling in normal pregnancy and for the pathogenesis of pregnancy complications such as pre-eclampsia and fetal growth restriction.

Materials and Methods

Reagents

Full details of reagents used are provided in the online data supplement available at http://circres.ahajournal.org.

Tissue, Cell Culture, and Labeling

Informed consent was obtained for all myometrial and placental tissue used in this study and ethical committee approval was in place. Normal first trimester placenta and decidua (8 to 13 weeks) was obtained at elective surgical termination of pregnancy. Term decidual/myometrial biopsies taken from nonplacental bed tissue were obtained from women with normal pregnancies at elective caesarean section. Isolation and culture of first trimester primary cytotrophoblast (CTB), and culture and labeling of human aortic SMC (HASMC) are described in the online data supplement.

Time-Lapse Microscopy

Apoptosis was monitored by time-lapse microscopy as described previously.[10,11] Images were analyzed using ImagePro Plus (Media Cybernetics, Silver Spring, Md). Details are provided in the online data supplement.

Immunoblotting

Preparation of HASMC lysates for analysis of cleaved PARP expression is described in the online data supplement.

Preparation of Spiral Artery Sections

Dissection of spiral arteries was performed as previously described.[10,11,31] Details are provided in the online data supplement.

Immunohistochemistry

Details of cell and tissue staining and microscopic analysis are provided in the online supplement.

Flow Cytometry

Flow cytometric analysis of TRAIL expression on trophoblast and TRAIL receptor expression on HASMC is described in the online supplement. Data were analyzed using WinMDI 2.8 freeware (http://facs.scipps.edu/software.html).

Vessel Explant Model

Dissection and perfusion of spiral arteries was performed as previously described.[10,11,31] Details are provided in the online data supplement.

TUNEL Staining

TUNEL staining of tissue sections was performed as previously described.[10] Details are provided in the online data supplement.

Measurement of TRAIL Expression

Lysates and medium from primary CTB were analyzed for TRAIL, as described in the online data supplement.

Statistics

Data were compared using either a repeated measures ANOVA or paired *t* test (parametric) or a Kruskal-Wallis test (nonparametric). Appropriate posthoc tests were applied and all statistical analyses performed using GraphPad Prism software, version 4 (GraphPad Software, San Diego, Calif). Significance was taken as $P<0.05$. Data are presented as the mean±SEM from at least 3 independent experiments.

Results

TRAIL Induces Smooth Muscle Cell Death

To investigate whether TRAIL can induce death of SMC, cultures of HASMC were treated with rhTRAIL and apoptosis was assessed. Using time-lapse microscopy, images of the cells were taken at 15 minute intervals over 65 hours. The time of onset of apoptosis, characterized by the first appearance of apoptotic morphology, was scored for 40 individual cells and a cumulative time course of apoptosis was derived. Recombinant human TRAIL (rhTRAIL) instigated apoptosis of the cells in a concentration-dependent manner over 65 hours (Figure 1A). The percent apoptotic cells at 60 hours was significantly increased by rhTRAIL at concentrations of 0.01 μg/mL and above (Figure 1B).

To confirm cell death in response to TRAIL occurred by apoptosis, HASMC cultures treated with rhTRAIL were lyzed and analyzed by immunoblotting for the expression of cleaved poly(ADP-ribose) polymerase (PARP), a marker of apoptosis. Following 24 or 60 hours treatment with 0.1 μg/mL rhTRAIL, a significant increase in cleaved PARP protein was observed (Figure 2A). To further verify that cell death was apoptotic, time-lapse analysis of rhTRAIL-treated cells was repeated in the presence of the pan caspase inhibitor zVAD-fmk. As caspases are central in the execution of programmed cell death, blocking caspase activation should rescue cells from apoptosis. After 60 hours treatment with rhTRAIL (0.5 μg/mL), the percent apoptotic HASMC was significantly increased. This increase was significantly inhibited by zVAD-fmk (Figure 2B). This data provides further evidence to suggest that HASMC death in response to rhTRAIL occurs via apoptosis.

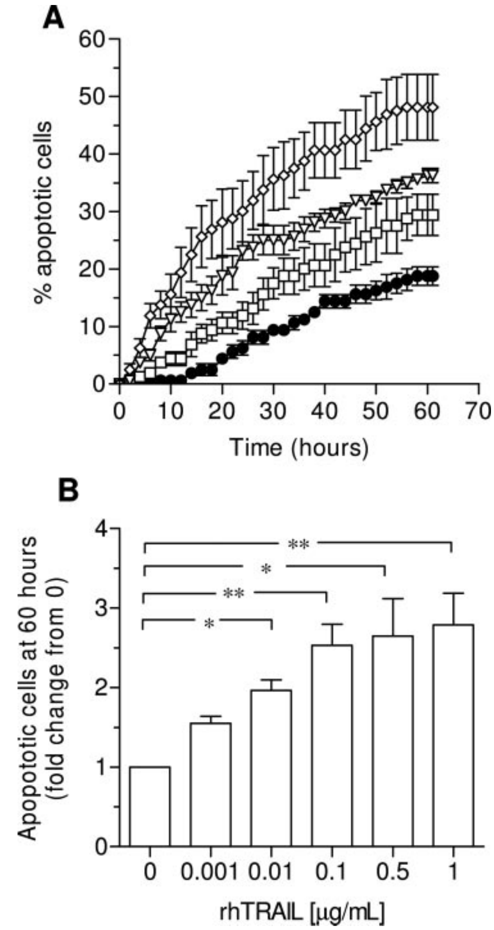

Figure 1. HASMC death is induced by rhTRAIL. HASMC were monitored by time-lapse microscopy and the time point at which cells underwent apoptosis was scored. A, Time course of HASMC apoptosis following stimulation with rhTRAIL: control (●), 0.001 μg/mL (□), 0.01 μg/mL (▽), 0.1 μg/mL (◇). B, % apoptotic HASMC at 60 hours, *$P<0.05$ and **$P<0.01$, Kruskal-Wallis test, Dunn's posthoc test (mean±SEM, n=4).

Receptor Expression and Involvement in TRAIL-Induced Apoptosis

TRAIL binds to five different receptors, two of which transduce an apoptotic signal. To investigate which receptors were involved in activating rhTRAIL-induced HASMC apoptosis, TRAIL-R1 and TRAIL-R2 expression on HASMC was first confirmed by flow cytometry (Figure 3A and B). Time-lapse microscopy was then used to assess HASMC apoptosis in the presence of antibodies that blocked activation of these receptors. Following stimulation with rhTRAIL (0.25 μg/mL) for 60 hours, HASMC apoptosis was significantly increased. In the presence of an antibody blocking either TRAIL-R1 or TRAIL-R2, rhTRAIL-induced apoptosis was significantly inhibited (Figure 3C and 3D, respectively). This indicates that both TRAIL-R1 and TRAIL-R2 are expressed on HASMC and that both are involved in apoptotic signaling.

As this study focuses on a role for TRAIL in vessel remodeling during pregnancy, it was necessary to demonstrate that receptors for TRAIL are expressed on spiral artery SMC in situ. Sections of first trimester decidua containing unmodified spiral arteries were stained using antibodies against TRAIL-R1 and -R2. Expression of both receptors was noted on a subset of spiral artery SMC (Figure 4A, B); receptor expression was also evident in the decidual stroma. Immunohistochemistry performed using an antibody against TRAIL indicated that first trimester extravillous trophoblast express this ligand in vivo (Figure 4C and 4D). These findings clarify that receptors for TRAIL are expressed on SMC in the placental bed, during the window when spiral artery remodeling takes place. At the same time, TRAIL-expressing trophoblasts are present within the decidua.

The Role of the Trophoblast in SMC Apoptosis

The aim of this work was to examine interactions between trophoblast and vascular cells, specifically SMC, to determine the role that trophoblast play in the loss of SMC during remodeling. By monitoring primary first trimester human cytotrophoblast (CTB) and HASMC cocultures by time-lapse microscopy, it was possible to examine these interactions.

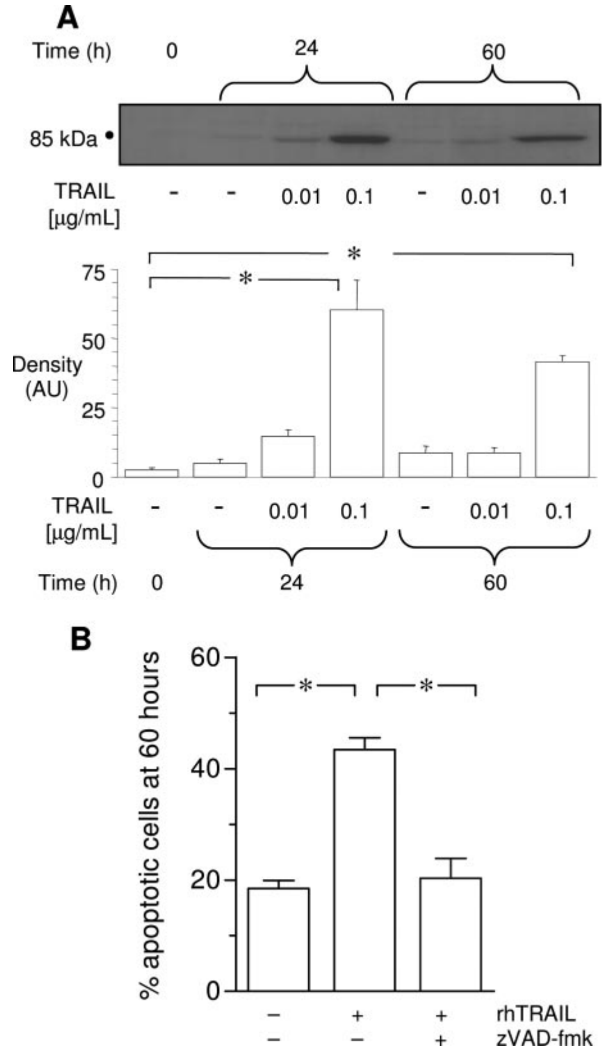

Figure 2. TRAIL-induced HASMC death occurs by apoptosis. A, Cultures of HASMC were treated with rhTRAIL (0.01 or 0.1 μg/mL) and apoptosis was assessed at 24 and 60 hours by immunoblot analysis of cleaved PARP expression. A representative auto-radiograph is shown. The bar chart shows the pooled densitometric data, *$P<0.001$, repeated measures ANOVA, Bonferroni's post-hoc test (mean±SEM, n=3). B, HASMC were treated with rhTRAIL (0.5 μg/mL) in the presence or absence of zVAD-fmk (50 μmol/L) and monitored by time-lapse microscopy. The time point at which cells underwent apoptosis was scored. % apoptotic HASMC at 60 hours is shown, *$P<0.001$, repeated measures ANOVA, Bonferroni's posthoc test (mean±SEM, n=4).

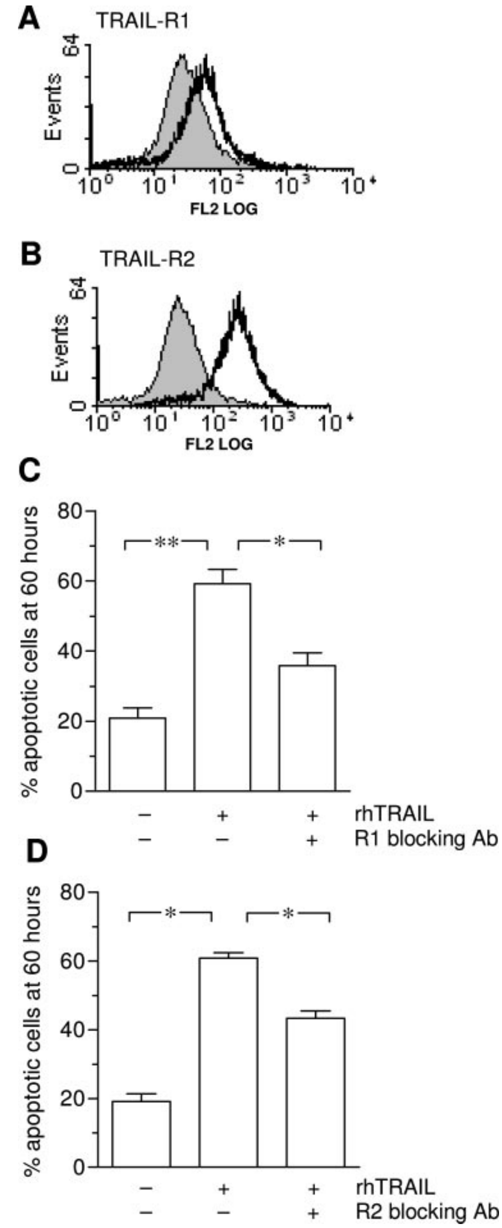

Figure 3. Expression of TRAIL receptors on HASMC and the effect of blocking TRAIL receptors on rhTRAIL-induced apoptosis. A and B, TRAIL receptor expression on HASMC assessed by flow cytometry. The histograms show unpermeablized cells labeled with isotype control IgG (gray shading) and (A) anti-TRAIL-R1 (open histogram) or (B) anti-TRAIL-R2 (open histogram). C and D, HASMC were monitored by time-lapse microscopy and the time point at which cells underwent apoptosis was scored. Shown is % apoptotic HASMC at 60 hours following stimulation with rhTRAIL (0.25 μg/mL) in the presence or absence of either (C) TRAIL-R1 blocking antibody (5 μg/mL) or (D) TRAIL-R2 blocking antibody (5 μg/mL). *$P<0.01$ and **$P<0.001$, repeated measures ANOVA, Bonferroni's posthoc test (mean±SEM, n=3).

A typical time-lapse sequence, highlighting a small segment of the total field of view, is shown in the accompanying video (http://circres.ahajournal.org). Six single images taken from this sequence are shown in Figure 5. It is apparent that direct contact is made between the two cell types, and that the primary CTB instigates this interaction (0 to 3 hours). As the primary CTB moves away (3 to 9 hours), a thin attachment extrudes out between the cells, indicated by the arrow. The HASMC then undergoes apoptosis, with a classic apoptotic blister forming after the primary CTB has departed (9 to 12 hours). Immunohistochemical staining of a parallel experiment using antibodies against smooth muscle actin (HASMC; red) and cytokeratin 7 (CTB; green) is also shown (Figure 5B–D). The time-lapse video sequence demonstrates that HASMC undergo apoptosis following interactions initiated by CTB, and that direct cell contact between the two cell types may be an important component of this process.

Time-lapse microscopy provides visual evidence for the involvement of CTB in HASMC apoptosis. Quantitative analysis of these sequences involved scoring the onset of apoptosis of HASMC in the presence or absence of primary

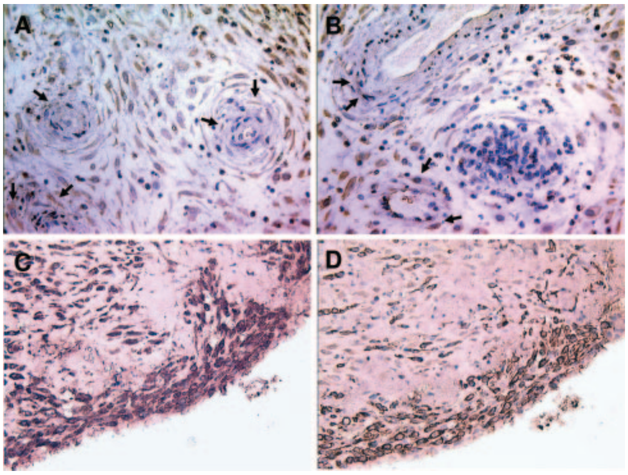

Figure 4. TRAIL and its receptors are expressed in first trimester placental bed tissue. Sections of wax-embedded first trimester decidua immunostained with antibodies against (A) TRAIL-R1, (B) TRAIL-*R2*, (C) TRAIL or (D) cytokeratin-7 (brown). Sections are counterstained with hematoxylin (blue). A and B, 11 weeks gestation; ← indicates positive cells. C and D, 12 weeks gestation. Pictures are representative of n=3 independent experiments.

CTB. The addition of primary CTB resulted in an increase in the rate of HASMC apoptosis over a 65 hour time course (Figure 6A). Comparison of the percent apoptotic cells at 60 hours shows a significant increase in HASMC apoptosis in the presence of primary CTB (Figure 6B).

Having demonstrated that the presence of primary CTB is sufficient to increase HASMC apoptosis, the involvement of TRAIL in this process was investigated. A construct consisting of the extracellular domain of recombinant human TRAIL-R1 (amino acids 24 to 239) fused to the Fc portion of human IgG_1 (rhTRAIL-R1:Fc) was used. The construct acts as a soluble receptor and binds all ligand (both soluble and membrane attached), thus preventing TRAIL from interacting with any of its receptors and inhibiting TRAIL-induced apoptosis. When this construct was added in time-lapse experiments to cocultures of HASMC, primary CTB-induced HASMC apoptosis was inhibited (Figure 6A). The percent of apoptotic HASMC in cocultures with primary CTB at 60 hours was significantly reduced following the addition of rhTRAIL-R1:Fc (Figure 6B). This provides persuasive evidence that TRAIL is involved in primary CTB-induced HASMC apoptosis.

TRAIL Production by Trophoblast

The data presented here suggest that SMC death is instigated by trophoblast by a mechanism involving the apoptotic cytokine TRAIL, however, the source of this cytokine is unclear. TRAIL may be produced by the trophoblast, or may originate from the SMC in an autocrine manner, possibly in response to a trophoblast-derived signal. In SMC monocultures, the rate of apoptosis was not significantly altered in the presence of the TRAIL-R1:Fc blocking construct or antibodies against TRAIL-R1 or R2, indicating that SMC-derived TRAIL does not contribute to basal SMC apoptosis in our system (% apoptotic cells at 60 hours: 26.7±3.6 (control) versus 25.0±2.5 (TRAIL-R1:Fc), 28.3±2.2 (TRAIL-R1), 40.0±4.3 (TRAIL-R2), NS, n=3). Furthermore, immunoblot analysis of HASMC lysates derived from noncontact cocultures with primary CTB showed no change in TRAIL expression (Figure 6C), and levels of TRAIL in the same SMC lysates, quantified by ELISA, were below the level of detection (detection limit 62.5 pg/mL). This data suggests that CTB do not stimulate TRAIL production by SMC.

We next sought to confirm that trophoblasts produce TRAIL. Cell lysates made from first trimester primary CTB cultured on Matrigel for 72 hours contained TRAIL in significant amounts, when measured by ELISA (572.4±160.3 pg TRAIL/mg protein, n=10). No TRAIL was detected in the medium collected from these cells. This suggested that TRAIL was expressed as the cell-associated form, rather than being cleaved and released in soluble form. To confirm this, flow cytometric analysis was performed on primary CTB cultured for 48 hours on Matrigel. Nonpermeablized first trimester primary CTB expressed TRAIL on the cell surface, confirming its presence as the membrane-bound form of this cytokine (Figure 6D).

TRAIL Causes SMC Apoptosis in Spiral Arteries

Having demonstrated using in vitro systems that SMC undergo apoptosis in response to either recombinant- or cell-derived TRAIL, we wished to confirm that the SMC present in spiral arteries were similarly sensitive to TRAIL-induced apoptosis. Denuded spiral arteries dissected from nonplacental bed biopsies were perfused with TRAIL (0.5 μg/mL), tied off and cultured for 24 hours. Vessels were fixed, sectioned and colabeled with TUNEL to detect apoptosis, and an antismooth muscle actin primary antibody to visualize SMC. Control vessels showed limited TUNEL-positive staining in the smooth muscle layers (Figure 7A, green staining), however, in vessels

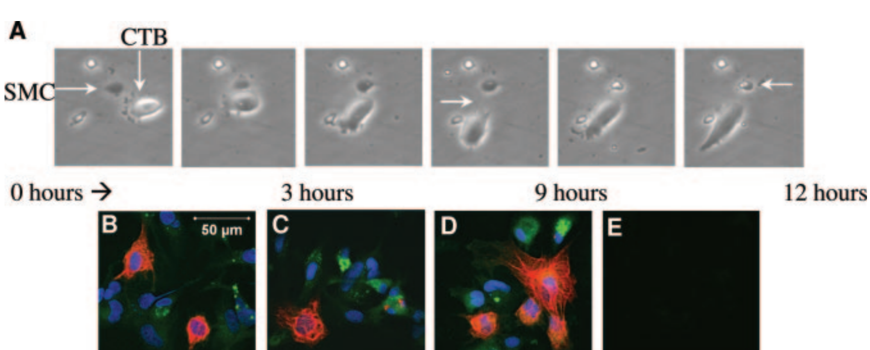

Figure 5. Apoptosis of a smooth muscle cell in the presence of primary CTB. A, Still pictures taken from a sequence of images of a coculture of primary trophoblast (CTB) and smooth muscle cells (SMC) 0 to 3 hours. Primary CTB approaches and interacts with SMC. 3 to 9 hours, trophoblast moves away. A thin attachment is visible (indicated by →). 9 to 12 hours; SMC undergoes apoptosis. A characteristic apoptotic blister is seen (indicated by ←). B–D, Immunohistochemical staining shows SMC staining positive for smooth muscle actin (red) and primary CTB staining positive for cytokeratin 7 (green). E, IgG control.

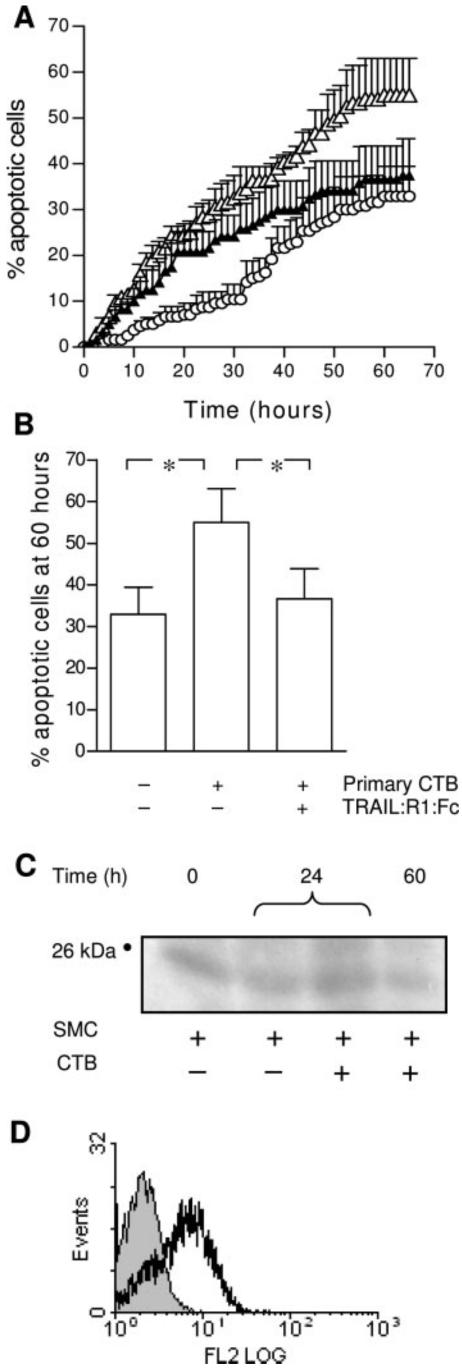

Figure 6. HASMC apoptosis induced by primary CTB is blocked by a TRAIL-R1:Fc construct and TRAIL is expressed by primary CTB and HASMC. A, Contact cocultures of HASMC alone (○) or HASMC and primary cytotrophoblasts (CTB, △) were treated with a TRAIL-R1:Fc construct (▲, as indicated) and then monitored by time-lapse microscopy. The time point at which cells underwent apoptosis was scored. A, Time course of HASMC apoptosis. B, % apoptotic HASMC after 60 hours of coculture. *$P<0.001$, repeated measures ANOVA, Bonferroni's posthoc test (mean±SEM, n=3). C, TRAIL expression by HASMC in the presence and absence of primary CTB was determined by immunoblot analysis. HASMC lysates were prepared at 24 and 60 hours from noncontact cocultures of primary CTB and HASMC. A representative autoradiograph is shown. D, Primary cytotrophoblast (CTB) 48 hours postisolation were assessed for cell-surface TRAIL expression by flow cytometry. The histograms show unpermeablized cells labeled with isotype control IgG (gray shading) or anti-hrTRAIL (open histograms). A representative from n=4 experiments is shown.

treated with TRAIL, significant apoptosis was observed in the smooth muscle layers (Figure 7B, C). Some TUNEL-positive staining was observed in the outer connective tissue of TRAIL-treated vessels, which may have been caused by disruption of the tissue architecture during dissection. Alternatively, apoptosis may have been induced in these cells by TRAIL-containing perfusate leaking into the bath before the ends of the vessels are tied off. To avoid the risk of contamination, control vessels are always perfused before the treated vessels. These data confirm that SMC present in the uterine spiral arteries are indeed susceptible to TRAIL-induced apoptosis, and support the role of trophoblast-derived TRAIL as a mediator of vascular SMC loss during spiral artery remodeling.

Discussion

In human pregnancy, the presence of the invading fetal trophoblast is a critical element that determines the outcome of spiral artery remodeling. Shallow or incomplete trophoblast invasion with limited vessel remodeling has been associated with complications such as preeclampsia,[5] preterm labor[3] and fetal growth restriction.[2] Some studies have focused on defects that lead to adverse outcomes, but little work has been done to understand the basis of remodeling in normal pregnancy. We have previously demonstrated that primary CTB and trophoblast cell lines can initiate endothelial and SMC apoptosis in spiral arteries via the Fas/Fas ligand pathway, and have proposed that a trophoblast-dependent apoptotic mechanism contributes to endothelial and SMC loss during remodeling in pregnancy.[10,11] The current study identifies TRAIL as another important apoptotic ligand produced by trophoblast, that is able to induce SMC apoptosis. Here we propose that activation of the TRAIL pathway also contributes to SMC loss during spiral artery remodeling in human pregnancy. Spiral artery endothelial cells may also be sensitive to TRAIL-induced apoptosis; indeed, a previous study has shown that endothelial cells express the activating TRAIL receptors.[28] Although not addressed in the current study, we have preliminary data showing that a human umbilical vein endothelial cell-derived cell line, SGHEC-7, undergoes apoptosis in response to TRAIL stimulation, and that spiral artery endothelial cells express TRAIL-R2 at term (Keogh and Cartwright 2006, unpublished observations).

The ability of TRAIL to cause SMC death by apoptosis is supported by several lines of evidence. Addition of rhTRAIL to HASMC cultures increased apoptosis in a concentration-dependent manner and was inhibited by pretreatment of cells with a pan caspase inhibitor. Furthermore, increased PARP cleavage was observed in response to rhTRAIL. In agreement with our findings, another study demonstated that incubation of HASMC with rhTRAIL for 24 hours induced significant apoptosis and PARP cleavage.[29] Other work has reported that TRAIL is without effect on human vascular SMC.[23] In this instance the concentrations of TRAIL used are comparable, however, the time of exposure was limited to 6 hours or less, which may be insufficient to see significant cell death. It has been reported that vascular SMC sequester death receptors within the cytoplasm and that basal cell surface expression is

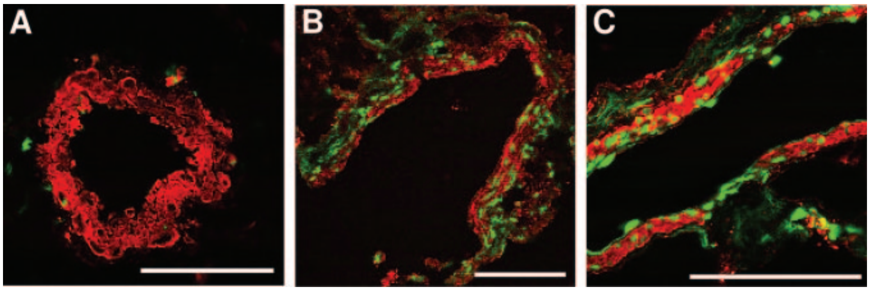

Figure 7. rhTRAIL induces apoptosis of arterial smooth muscle cells in situ. Sections from a denuded spiral artery perfused with (A) Control medium or (B and C) 0.5 μg/mL rhTRAIL. Vessels were tied off following perfusion and cultured for 24 hours before being frozen, cryosectioned and stained. TUNEL-positive cells are labeled with FITC (green) and medial SMC are stained for smooth muscle actin (red). Scale bar=100 μm. Pictures are representative of n=3 independent experiments.

relatively low.[32] Prolonged exposure to an apoptotic ligand may be required to allow sufficient receptors to be trafficked to, or expressed on, the cell surface to see a significant increase in apoptosis. A recent article has demonstrated that SMC are sensitized to Fas L-stimulated apoptosis by IFN-γ, which causes Fas to be trafficked to the cell surface.[33]

Another important consideration is that apoptosis is a slow process involving individual cells and thus proceeds in an asynchronous manner. DNA fragmentation and changes in the cellular ultrastructure are estimated to occur at least 12 to 14 hours from an initial stimulus.[34] End point analysis such as TUNEL staining may only capture a few apoptotic cells at any one point, giving an impression that TRAIL has no effect. By using time-lapse microscopy, a cumulative picture of how apoptosis proceeds can be obtained. While at any single time only a few cells may undergo apoptosis, by following a population it is possible to build a time course that shows the true effect of TRAIL, in this case on HASMC death. Our observation that TRAIL-R1 and -R2 are only expressed in a subset of spiral artery SMC in the first trimester placental bed imposes further spatial and temporal restrictions on TRAIL-induced apoptosis, thus helping to maintain vessel integrity during the remodeling process.

TRAIL was identified as a candidate for involvement in trophoblast-induced SMC apoptosis as it is produced by trophoblast. TRAIL protein immunoreactivity was detected in the cytotrophoblast, in sections from human placenta at 8 weeks gestation,[14] and in the syncytiotrophoblast of first trimester placenta.[15] TRAIL mRNA has been detected in the Jar and JEG-3 trophoblast cell lines,[15] however, another study by this group failed to detect a TRAIL mRNA transcript in primary CTB,[16] in contrast to our own data. Two differences in the cell isolation procedure may have contributed to this result. We isolated first trimester CTB and cultured them on Matrigel to promote differentiation to an extravillous phenotype, whereas Phillips et al measured TRAIL mRNA immediately postisolation in CTB isolated from term placenta.

The ELISA and flow cytometry data demonstrate that TRAIL is expressed as the cell-associated form in first trimester CTB. An additional observation in support of this is the direct interaction preceding apoptosis of HASMC, seen in the video sequence and the images captured from it. As apoptotic ligands such as TRAIL and FasL can be membrane bound or soluble, this suggests that, in the case of TRAIL, the cell-associated form may be the predominant form involved in initiating apoptosis. It is possible that the soluble form may be liberated in vivo and make a contribution to TRAIL-dependent apoptosis. Cleavage of TRAIL is initiated by cysteine proteases, of which the vessel wall is a rich source.[35] When trophoblast encounter SMC in the vessel wall, it is therefore possible that there is a localized release of soluble TRAIL. Furthermore, the results presented do not exclude the possibility that in utero, CTB may stimulate resident vascular cells to produce apoptotic factors, nor do they exclude the possibility that other cells present within the placental bed, such as macrophages or uterine natural killer cells, may release soluble TRAIL that could contribute to SMC apoptosis and vessel remodeling.

An immunological role for TRAIL in human pregnancy is the only one described to date. The feto-placental unit is a semi-allograft which must use mechanisms to prevent attack from maternal immune cells. The killing of activated lymphocytes by trophoblast via a TRAIL-dependent mechanism has been proposed to contribute to the attainment of immune tolerance by the fetus.[15,36] We propose that another physiological role in pregnancy can now be assigned to TRAIL, that is, an involvement in the mechanisms underlying remodeling of uterine spiral arteries.

This study shows for the first time that primary first trimester CTB and extravillous trophoblast in situ express the apoptotic cytokine TRAIL, and that CTB use the TRAIL pathway to induce SMC apoptosis. These findings are significant on two levels. First, the results demonstrate a role for TRAIL under normal physiological conditions. This is important as the potential use of TRAIL as a therapeutic agent relies on detailed knowledge of its actions in a nonpathological state. Secondly, in addition to its immunological role, a new function can be ascribed to TRAIL in human pregnancy, that is, as a contributor to SMC loss during remodeling. The demonstration that first trimester CTB use a TRAIL-dependent mechanism to induce SMC death further defines the mechanisms that underlie vessel remodeling in normal pregnancies. Elucidating the fundamental elements of this remodeling process is critical, because defects in vessel remodeling have been identified in pathological complications of pregnancy that compromise both maternal and fetal health. To develop treatments for compromised pregnancies it is essential to understand the basis of the vascular changes that occur in normal pregnancy.

Sources of Funding

This work was supported by a grant from the Wellcome Trust, UK (069939).

Disclosures

None.

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