

# Abnormal Placentation and the Syndrome of Preeclampsia

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Preeclampsia, particularly the severe cases that occur early in pregnancy, is associated with defects in the (placental) cytotrophoblast differentiation pathway that leads to uterine invasion. At a morphologic level, interstitial invasion often is shallow. Perhaps more significantly, endovascular invasion, particularly the arterial component, is rudimentary. The latter defect is thought to lead to hypoperfusion of the placenta. At a molecular level, these defects are associated with particular deficits in the differentiation process whereby cytotrophoblasts—epithelial cells of ectodermal origin—assume vascular-like properties. Until recently, the question was how the latter defects could lead to the maternal symptoms of this condition. Now a possible link in the form of preeclampsia-associated changes in placental production of vasculogenic/angiogenic substances and their inhibitors has been discovered. It is likely that this new paradigm will improve both diagnosis and treatment of this life-threatening pregnancy complication.

*Semin Nephrol* 24:540–547 © 2004 Elsevier Inc. All rights reserved.

**KEYWORDS** preeclampsia, placenta, cytotrophoblast, spiral arteries, angiogenesis

The placenta is a transient organ that is depicted commonly as a simple, pancake-shaped sponge connecting the embryo/fetus to the uterus. Before birth, this portion of the placenta performs many metabolic and transport activities. But some of the placenta's most novel and interesting functions lie in a part of this organ that rarely appears in diagrams—the maternal/fetal interface. Here cytotrophoblasts (CTBs) that arise from its surface attach the conceptus to the mother by invading the uterus. Integral components include the targeted migration of CTBs, a process governed by intricately patterned interactions with maternal tissue including the uterine vasculature. In the following sections we discuss, first at a cellular and then at a molecular level, the interactions that occur between invasive (fetal) CTBs and uterine (maternal) cells during normal pregnancy. The importance of these events is highlighted by the specific placental defects that are associated with the pregnancy-specific complication of preeclampsia.

## Cellular Aspects of CTB Invasion in Normal Pregnancy

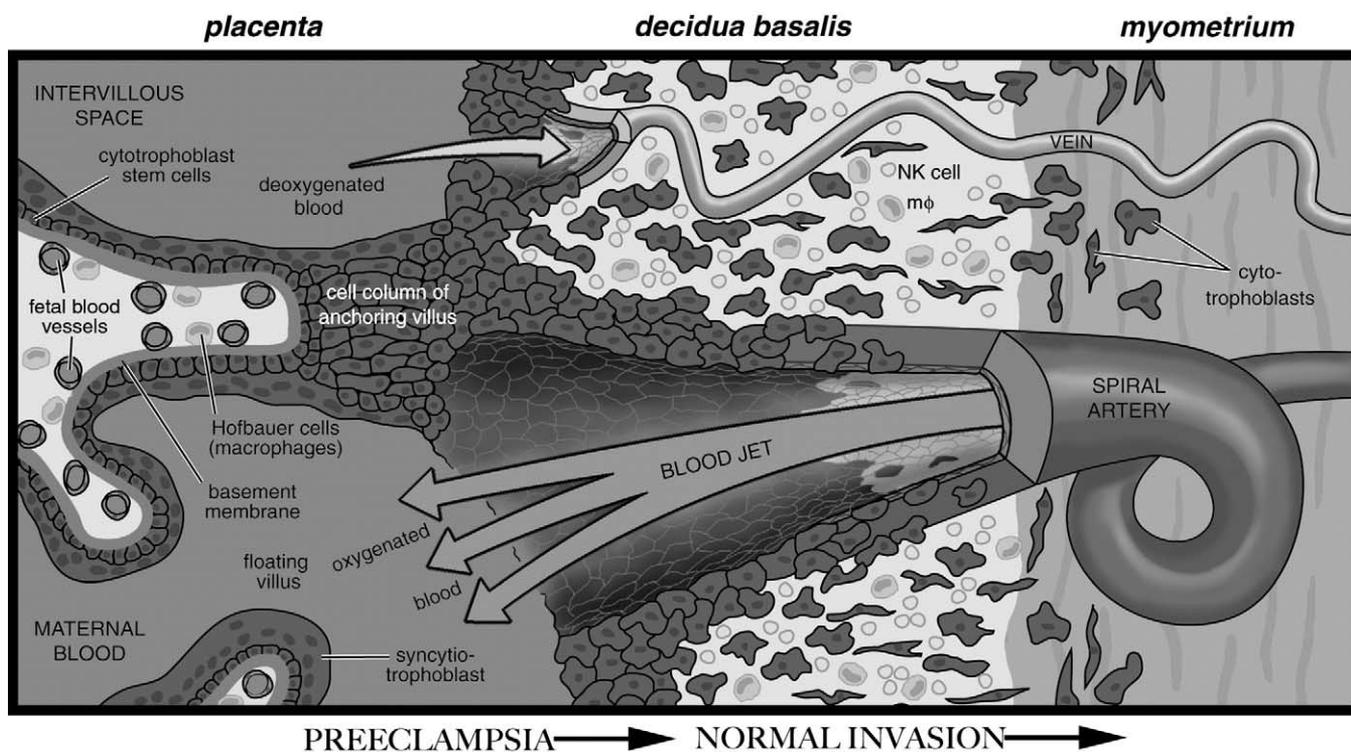
Development of the human maternal-fetal interface presents an interesting opportunity to study seemingly unique processes. For example, uterine attachment requires the organ's specialized epithelial cells, termed CTBs, to acquire the ability to invade maternal tissues aggressively.<sup>1,2</sup> Key morphologic aspects of the invasion process are diagrammed in Figure 1. The progenitor (fetal) stem cells form a polarized epithelium that is attached to the basement membrane surrounding the stromal cores of chorionic villi. During differentiation along the invasive pathway, CTBs leave this basement membrane to form columns of unpolarized cells that attach to and then penetrate the uterine wall. The ends of the columns terminate within the superficial endometrium, where they give rise to invasive (extravillous) CTBs. During interstitial invasion, a subset of these cells, either individually or in small clusters, commingles with resident decidual, myometrial, and immune cells. During endovascular invasion, masses of CTBs breach and plug the vessels (likened to dripping candle wax<sup>3</sup>). Subsequently, these fetal cells replace the resident maternal endothelium and portions of the smooth muscle wall, creating a novel hybrid vasculature composed of both maternal and fetal cells. Normally, this

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Supported in part by grants from the National Institutes of Health (HD30367 and HL64597) and the Tobacco-Related Disease Research Program (9RT-0112).

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**Figure 1** Diagram of the histologic organization of the human maternal-fetal interface at midgestation. In this location, CTBs, specialized (fetal) epithelial cells of the placenta, differentiate and invade the uterine wall, where they also breach maternal blood vessels. The basic structural unit of the placenta is the chorionic villus, composed of a stromal core with blood vessels, surrounded by a basement membrane, and overlaid by cytotrophoblast progenitors. As part of their differentiation program, these cells detach from the basement membrane and adopt 1 of 2 lineage fates. They either fuse to form the syncytiotrophoblasts that cover floating villi, or join a column of extravillous cytotrophoblasts at the tips of anchoring villi. The syncytial covering of floating villi mediates the nutrient, gas, and waste exchange between fetal and maternal blood. The anchoring villi, through the attachment of cytotrophoblast columns, establish physical connections between the fetus and the mother. Invasive cytotrophoblasts penetrate the uterine wall up to the first third of the myometrium. A portion of the extravillous cytotrophoblasts home to uterine spiral arterioles and remodel these vessels by destroying the muscular wall and replacing the endothelial lining. To a lesser extent they also remodel uterine veins. At term, few cytotrophoblast progenitors remain, the syncytiotrophoblast layer thins and the stromal cores expand. As indicated by the arrows at the bottom of the diagram, invasion normally extends into the myometrium. In contrast, in preeclampsia invasion is relatively more shallow. Reprinted from Zhou et al<sup>16</sup> with permission.

process extends throughout the portions of uterine arterioles that span the decidua and the inner third of the myometrium (see Fig 1). In contrast, only the termini of uterine veins are breached.<sup>4</sup> Together the 2 components of CTB invasion anchor the placenta to the uterus and permit a steady increase in the supply of maternal blood that is delivered to the growing fetus. Equally unusual is the fact that placental development takes place in a specialized local environment that induces maternal immunologic tolerance of the semiallogeneic fetal CTBs.<sup>5,6</sup>

## Molecular Aspects of Cytotrophoblast Differentiation in Normal Pregnancy

Some of the key molecular aspects of human CTB differentiation and invasion are known. The cells' expression of several classes of functionally relevant molecules is precisely modu-

lated as they invade either in situ (the uterine wall) or in vitro (extracellular matrix, eg, Matrigel, (BD Biosciences, Bedford, MA)). Overall, invasive cytotrophoblasts mimic broadly the adhesion phenotype of the endothelial cells they replace, and these changes have the net effect of enhancing CTB motility and invasiveness.<sup>7</sup> To examine individual components of this phenomenon, we first stained tissue sections of the fetal-maternal interface for specific integrins, cadherins, and immunoglobulin family adhesion receptors that are characteristic of vascular cells. Subsequent experiments tested the functional consequences of inhibiting the activity of particular adhesion receptors that were up-regulated during CTB differentiation.

For example, we examined the distribution patterns of  $\alpha V$  integrin family members. These molecules are of particular interest because of their regulated expression on endothelial cells during angiogenesis and their up-regulation on some types of metastatic tumor cells.<sup>8,9</sup>  $\alpha V$  family members dis-

played unique and highly specific spatial staining patterns on CTBs in anchoring villi and the placental bed. An antibody specific for the  $\alpha V\beta 5$  complex stained the CTB monolayer in chorionic villi. Staining was uniform over the entire cell surface. The syncytiotrophoblast layer, and CTBs in cell columns and the placental bed, did not stain for  $\alpha V\beta 5$ . In contrast, anti- $\alpha V\beta 6$  stained only those chorionic villus CTBs that were at sites of column formation. The CTB layer still in contact with the basement membrane stained brightly, whereas the first layer of the cell column showed reduced staining. The rest of the CTBs in chorionic villi, CTBs in more distal regions of cell columns, and CTBs within the placental bed and vasculature did not stain for  $\alpha V\beta 6$ , documenting a specific association of this integrin with initiation of column formation. In yet a different pattern, staining for anti- $\alpha V\beta 3$  was weak or not detected on villus CTBs or on CTBs in the initial layers of cell columns. However, strong staining was detected on CTBs within the uterine wall and vasculature. Thus, individual members of the  $\alpha V$  family, like those of the  $\beta 1$  family,<sup>10</sup> are regulated spatially during CTB differentiation. Of particular relevance is the observation that  $\alpha V\beta 3$  integrin, whose expression on endothelial cells is stimulated by angiogenic factors, is prominent on CTBs that have invaded the uterine wall and maternal vasculature.

Because blocking  $\alpha V\beta 3$  function suppresses endothelial migration during angiogenesis, we determined whether perturbing its interactions also affects CTB invasion in vitro. Freshly isolated first-trimester CTBs were plated for 48 hours on Matrigel-coated Transwell (Costar, Corning, NY) filters in the presence of control mouse immunoglobulin (Ig)G or the complex-specific anti- $\alpha V\beta 3$  IgG, LM609. CTB invasion was evaluated by counting cells and cellular processes that had invaded the Matrigel barrier and extended through the holes in the Transwell filters. LM609 reduced CTB invasion by more than 75% in this assay, indicating that this receptor, similar to the  $\alpha 1\beta 1$  integrin,<sup>7</sup> contributes significantly to the invasive phenotype of CTBs.

Next, we examined cadherin switching during CTB differentiation in vivo. The CTB epithelial monolayer stained strongly for the ubiquitous epithelial cell-cell adhesion molecule, E-cadherin, in a polarized pattern. Staining was strong on the surfaces of CTBs in contact with one another and with the overlying syncytiotrophoblast layer, and was absent at the basal surface of CTBs in contact with the basement membrane. In cell columns, E-cadherin staining intensity was reduced on CTBs near the uterine wall and on CTBs within the decidua. This reduction in staining was particularly pronounced in second-trimester tissue. At this stage, E-cadherin immunoreactivity also was very weak or undetectable on CTBs that had colonized maternal blood vessels and on CTBs in the surrounding myometrium. All locations of reduced E-cadherin staining were areas in which invasion is active during the first half of gestation. Interestingly, the staining intensity of E-cadherin was strong on CTBs in all locations in term placentas, when CTB invasive activity largely was absent. Taken together, these data are consistent with the idea that CTBs transiently decrease E-cadherin function at times and places of their greatest invasive activity.

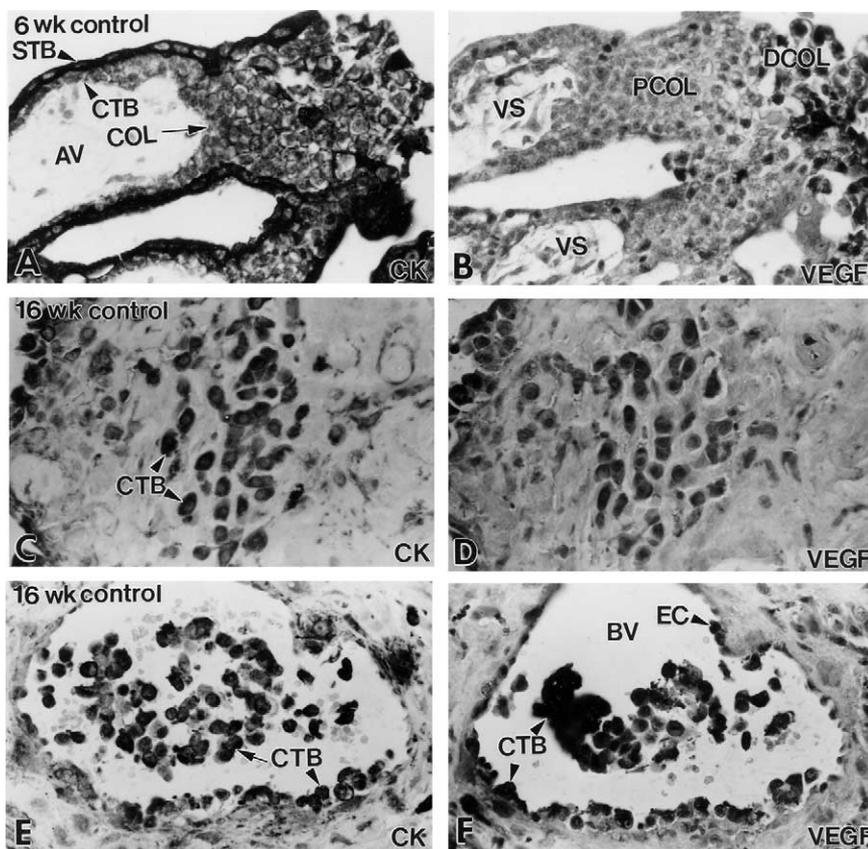
Frequently, cadherin switching occurs during embryonic development when significant morphogenetic events take place. We therefore stained sections of first- and second-trimester placental tissue with antibodies to other classic cadherins. These tissues did not react with antibodies against placental cadherin, but did stain with 3 different monoclonal antibodies that recognize the vascular endothelial cadherin, VE-cadherin. In chorionic villi, antibody to VE-cadherin did not stain villus CTBs, although it stained the endothelium of fetal blood vessels within the villus stroma. In contrast, anti-VE-cadherin stained CTBs in cell columns and in the decidua, the very areas in which E-cadherin staining was reduced. VE-cadherin staining was stronger in these areas in second-trimester tissues. In maternal vessels that had not yet been modified by CTBs, anti-VE-cadherin stained the endothelial layer strongly. After endovascular invasion, CTBs lining maternal blood vessels also stained strongly for VE-cadherin. Thus, CTBs that invade the uterine wall and vasculature express a cadherin characteristic of endothelial cells.<sup>4</sup>

Next, we used function-perturbing anticadherin antibodies, in conjunction with the Matrigel invasion assay, to assess the functional consequences of cadherin modulation by placental cells. We plated isolated second-trimester CTBs for 48 hours on Matrigel-coated filters in the presence of control IgG or function-perturbing antibodies against VE-cadherin or E-cadherin. By 48 hours, significant invasion was evident in control CTBs. In cultures treated with anti-E-cadherin, CTB invasiveness increased more than 3-fold, suggesting that E-cadherin normally has a restraining effect on invasiveness. In contrast, antibody against VE-cadherin decreased the invasion of CTBs to about 60% of control values. This suggests that the presence of VE-cadherin normally facilitates CTB invasion. Taken together, these functional data support the concept that as they differentiate, the cells modulate their cadherin repertoire to increase their invasiveness.

Our data presented thus far indicate that, as they differentiate, CTBs down-regulate adhesion receptors highly characteristic of epithelial cells (integrin  $\alpha 6\beta 4$ <sup>10</sup> and E-cadherin) and up-regulate analogous receptors that are expressed on endothelial cells (integrins  $\alpha 1\beta 1$ <sup>10</sup> and  $\alpha V\beta 3$ , and VE-cadherin). These observations show that normal CTBs undergo a comprehensive switch in phenotype so as to resemble the endothelial cells they replace during endovascular invasion.

We hypothesize that this unusual phenomenon plays an important role in the process whereby these cells form vascular connections with the uterine vessels. Ultimately, these connections are so extensive that the spiral arterioles become hybrid structures in which fetal CTBs replace the maternal endothelium and much of the highly muscular tunica media.<sup>11-13</sup> As a result, the diameter of the spiral arterioles increases dramatically, allowing blood flow to the placenta to keep pace with fetal growth. Circumstantial evidence suggests that several of the adhesion molecules whose expression we studied could play an important role in forming these novel vascular connections. In the mouse, for example, targeted disruption of either the vascular cell adhesion molecule-1 or  $\alpha 4$  integrin expres-

**Figure 2** CTB staining for VEGF-A was up-regulated as the cells differentiated and invaded the uterus in situ. Serial paraffin sections of the maternal-fetal interface were stained with (A, C, and E) anticytokeratin (CK) to identify all of the trophoblast populations, and with (B, D, and F) anti-VEGF-A. Essentially the same staining pattern was observed during the first and second trimesters (ie, 6 and 16 weeks of gestation). (B) A few CTB progenitors and cells in the proximal column (PCOL) region stained with anti-VEGF-A, (D) but much more intense staining was observed in association with a majority of CTBs in the distal regions of columns (DCOL) and with those that invaded the uterine wall. (F) CTBs within the lumina of uterine blood vessels (BV) also exhibited intense staining (arrowheads), as did the maternal endothelial cells (EC). In contrast, some CTBs in the vessel wall failed to react with anti-VEGF-A (arrows). The cells continued to stain for VEGF-A at term (see Fig. 3). AV, anchoring villus; STB, syncytiotrophoblast; VS, villous stroma; COL, cytotrophoblast column. Reprinted from Zhou et al<sup>16</sup> with permission.



sion results in failed chorioallantoic fusion.<sup>14</sup> It is very interesting to note that CTBs are the only cells, other than the endothelium, that express VE-cadherin. In addition, VE-cadherin and platelet-endothelial cell adhesion molecule-1 are the first adhesion receptors expressed by differentiating endothelial cells during early development as shown by Risau and Flamme.<sup>15</sup>  $\alpha V\beta 3$  expression is up-regulated on endothelial cells during angiogenesis by soluble factors that regulate this process. Thus, adhesion receptors that are up-regulated as the normal CTBs differentiate and invade, play vital roles in differentiation and expansion of the vasculature.

Given that CTBs have the unusual ability to mimic the cell-surface properties of endothelial cells, we went on to ask whether they also express molecules that play important regulatory roles in conventional vasculogenesis and/or angiogenesis,<sup>16</sup> principally vascular endothelial growth factor (VEGF) family members. By using a combination of in situ and in vitro approaches, we showed that CTB differentiation and invasion during the first and second trimesters of pregnancy were associated with down-regulation of VEGF receptor (VEGFR)-2. Invasive CTBs in early gestation expressed VEGF-A (Fig. 2), VEGF-C, placental growth factor (PlGF), VEGFR-1, and VEGFR-3, and, at term, VEGF-A, PlGF, and VEGFR-1. In vitro, the cells incorporated VEGF-A into the surrounding extracellular matrix; PlGF was secreted. We also found that CTBs responded to the VEGF ligands they produced. Blocking

ligand binding significantly decreased the cells' ability to invade owing to a large increase in apoptosis.

## Cellular Aspects of Cytotrophoblast Invasion in Preeclampsia

Preeclampsia is a disease that adversely affects 7% to 10% of first pregnancies in the United States.<sup>17</sup> The mother shows signs and symptoms that suggest widespread alterations in endothelial function (eg, high blood pressure, proteinuria, and the rapid accumulation of edema<sup>18</sup>). In some cases, fetal growth slows, which leads to fetal growth restriction. The severity of the disease varies greatly. In its mildest form the signs/symptoms appear near term and resolve after birth, with no lasting effects on either the mother or the child. In its severest form the signs/symptoms often occur in the second or early third trimesters. If they cannot be controlled, the only option is delivery, with consequent iatrogenic fetal prematurity. Owing to the latter form of the disease, preeclampsia and hypertensive diseases of pregnancy are leading causes of maternal death and contribute significantly to premature deliveries in the United States.<sup>18</sup>

Although the cause of preeclampsia is unknown, the accumulated evidence strongly implicates the placenta.<sup>19</sup> Anatomic examination shows that the area of the placenta

most affected by this syndrome is the fetal-maternal interface. CTB invasion of the uterus is abnormal, particularly in the severe form of the disease, where endovascular invasion beyond the terminal portions of the spiral arterioles is decreased.<sup>13,20</sup> The effect of preeclampsia on endovascular invasion is particularly evident when interactions between fetal CTBs and maternal endothelial cells are studied in detail.<sup>4,21</sup> Serial sections through placental bed biopsies of all the patients we have studied shows that few of the spiral arterioles contained CTBs. Instead, most CTBs remained at some distance from these vessels. Where endovascular CTBs are detected, their invasion is limited to the portion of the vessel that spans the superficial decidua. Thus, there is little difference between CTB interactions with veins and arterioles in the uterus. Even if the CTBs gain access to the lumen, they usually fail to form tight aggregates among themselves, or to spread out on the vessel wall, as is observed for CTBs in control samples matched for gestational age. Instead, they tend to remain as individual rounded cells, suggesting that they are anchored poorly to the vessel wall. Thus, CTBs in preeclampsia not only have a limited capacity for endovascular invasion but also display alterations, at a morphologic level, in their interactions with maternal arterioles.

Because of these alterations in endovascular invasion, the maternal vessels of preeclamptic patients do not undergo the complete spectrum of physiologic changes that normally occur (eg, loss of endothelial lining and musculoelastic tissue); the mean external diameter of the myometrial vessels is less than half that of similar vessels from uncomplicated pregnancies.<sup>20,22,23</sup> In addition, not as many vessels show evidence of CTB invasion.<sup>20,22,24</sup> Thus, the architecture of these vessels precludes an adequate response to gestation-related fetal demands for increased blood flow.

## Molecular Aspects of Cytotrophoblast Differentiation in Preeclampsia

We used our knowledge of the molecular aspects of CTB differentiation/invasion in normal pregnancy to test the hypothesis that preeclampsia is associated with specific deficits in this process. In this regard, the cells' adhesion molecule repertoire is particularly informative.<sup>21</sup> Briefly, we compared CTB expression of the aforementioned  $\alpha V$  family members in placental bed biopsy specimens obtained from control and preeclamptic patients that were matched for gestational age. Preeclampsia changed CTB expression of all 3  $\alpha V$ -family members. When samples were matched for gestational age, fewer preeclamptic CTB progenitors stained with an antibody that recognized integrin  $\beta 5$ . In contrast, staining for  $\beta 6$  was much brighter in preeclamptic tissue and extended beyond the column to include CTBs within the superficial decidua. Interestingly, staining for  $\beta 3$  was weak on CTBs in all locations; CTBs in the uterine wall of preeclamptic patients failed to show strong staining for  $\beta 3$ , as did CTBs that pene-

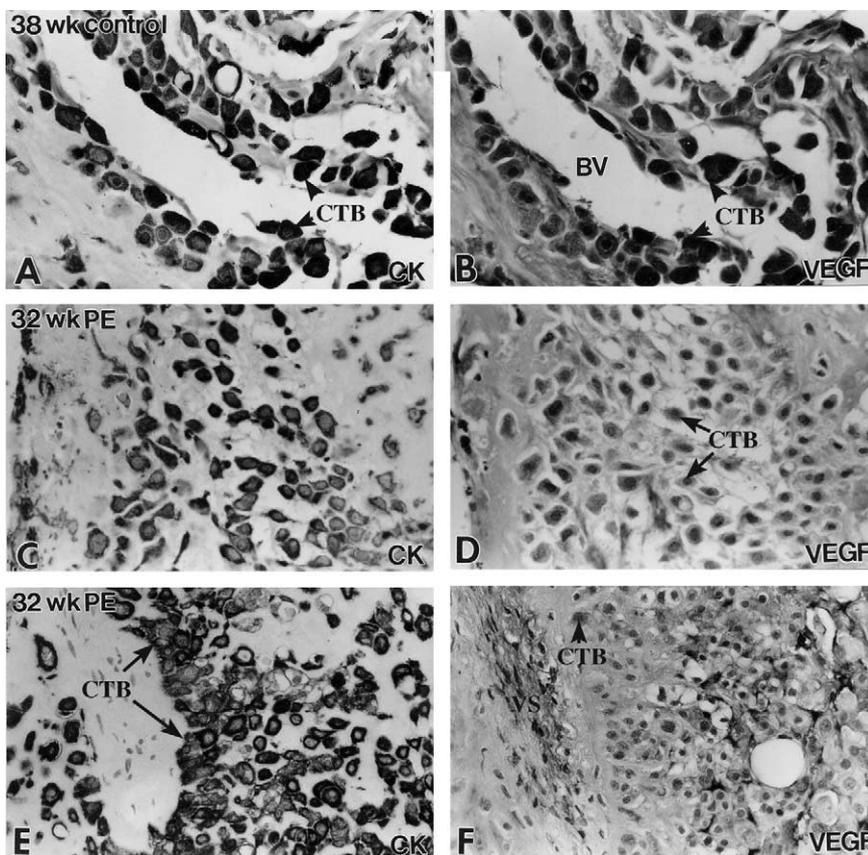
trated the spiral arterioles. Thus, in preeclampsia, differentiating/invasive CTBs retain expression of  $\alpha V\beta 6$ , which is expressed transiently in remodeling epithelium, and fail to up-regulate  $\alpha V\beta 3$ , which is characteristic of angiogenic endothelium. Therefore, as was the case for other CTB-expressed integrins,<sup>25</sup> our analyses of the expression of  $\alpha V$ -family members suggest that in preeclampsia, CTBs start to differentiate along the invasive pathway but cannot complete this process.<sup>21</sup>

Preeclampsia also had a striking effect on CTB cadherin expression. In contrast to control samples, CTBs in both the villi and decidua showed strong reactivity with anti-E-cadherin, and staining remained strong even on CTBs that had penetrated the superficial portions of uterine arterioles. Interestingly, in preeclampsia, CTBs within the uterine wall tended to exist as large aggregates, rather than as smaller clusters and single cells, as is the case in normal pregnancy. This observation is in accord with the likelihood that E-cadherin mediates strong intercellular adhesion among CTBs, as it does in all other normal epithelia examined.

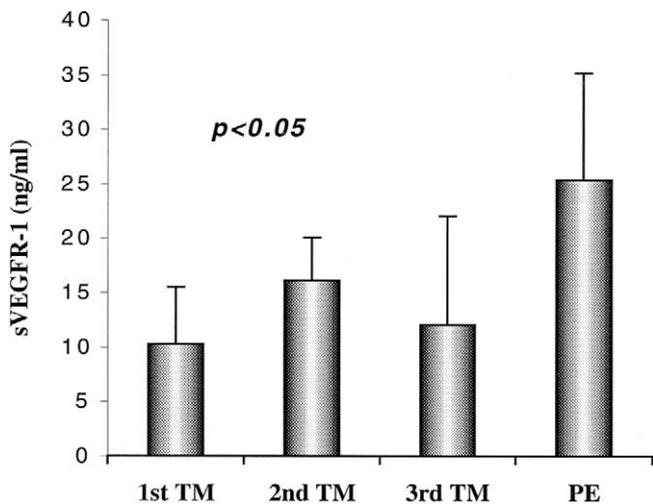
Strikingly, no VE-cadherin staining was detected on CTBs in any location in placental bed specimens obtained from preeclamptic patients; neither CTBs in the cell columns nor the few cells that were found in association with vessels in the superficial decidua expressed VE-cadherin. However, staining for this adhesion molecule was detected on maternal endothelium in the unmodified uterine vessels in preeclamptic placental bed biopsy specimens. Thus, cadherin modulation by CTBs in preeclampsia was defective, as shown by the persistence of strong E-cadherin staining and the absence of VE-cadherin staining on CTBs in columns and in the superficial decidua.

Is preeclampsia also associated with changes in CTB expression of VEGF family members? We used immunolocalization techniques to characterize the cells' expression of these ligands and receptors in tissue sections of placental bed biopsy specimens obtained from pregnant women with severe forms of preeclampsia that necessitated delivery during the early third trimester. The results showed that CTB VEGF-A and VEGFR-1 staining decreased (Fig. 3), whereas staining for PlGF was unaffected. CTB secretion of the soluble form of VEGFR-1 in vitro also increased (Fig. 4). Together, the results of this study showed that CTB expression of VEGF family members is misregulated in severe forms of preeclampsia.

In summary, our results raise the possibility that the failure of preeclamptic CTBs to express vascular-type adhesion molecules and growth factors, as normal CTBs do, impairs their ability to form connections with the uterine vessels. This failure ultimately limits the supply of maternal blood to the placenta and fetus, an effect thought to be linked closely to the pathophysiology of the disease. We also hypothesize that the failure of preeclamptic CTBs to make a transition to a vascular cell adhesion phenotype might be part of a broader-spectrum defect in which the cells fail to function properly as endothelium. Such a failure would no doubt have important effects on the maintenance of vascular integrity at the maternal-fetal interface. Clearly, in preeclampsia undifferentiated



**Figure 3** In preeclampsia, staining of invasive cytotrophoblasts with anti-VEGF-A decreased. (B) In the third trimester of normal pregnancy (38 weeks), CTBs in the interstitium (arrow) and in the walls of blood vessels (BVs) (arrowheads) stained for VEGF-A. (D and F) In preeclampsia (PE), staining of invasive CTBs for VEGF-A was strikingly down-regulated. CK, cytokeratin (A, C and E); VS, villus stroma. Reprinted from Zhou et al<sup>16</sup> with permission.



**Figure 4** In preeclampsia, CTBs produce higher levels of soluble VEGFR-1 (sVEGFR-1) in vitro as compared with control cells. CTBs were isolated from control first-, second-, or third-trimester (TM) placentas or from the placentas of women whose pregnancies were complicated by preeclampsia (PE). Then the cells were cultured on Matrigel substrates for 48 hours. Quantification of sVEGFR-1 in the conditioned medium by enzyme-linked immunosorbent assay showed that preeclampsia is associated with a significant increase of CTB sVEGFR-1 secretion as compared with control cells. Data are means and SDs of 5 experiments.  $P < .05$ . Reprinted from Zhou et al<sup>16</sup> with permission.

CTBs that fail to mimic an endothelial cell phenotype are present in the termini of maternal spiral arterioles. Whether their presence affects the phenotype of maternal endothelium in deeper segments of the same vessels and/or is linked to the maternal endothelial pathology that is a hallmark of this disease remains to be investigated.

### Conclusions

We now understand a great deal about CTB defects in the placentas of patients whose pregnancies are complicated by preeclampsia. In a landmark study published over 30 years ago, Brosens et al<sup>20</sup> were among the first to describe the abnormally shallow CTB invasion that is observed in preeclampsia and a substantial proportion of pregnancies complicated by fetal growth restriction. These investigators considered the lack of invasion of the spiral arterioles to be particularly significant. Building on this foundation, our recent studies have shown that CTB invasion of the uterus is actually a unique differentiation pathway in which the fetal cells adopt certain attributes of the maternal endothelium they normally replace. In preeclampsia, this differentiation process goes awry.

Currently, we are very interested in using these findings as a point of departure for studies of the disease process from its inception to the appearance of the maternal signs. With regard to its inception, understanding the nature of the phenotypic alterations that are characteristic of CTBs in preeclampsia offers us the exciting opportunity to test hypotheses about

the causes. From a reductionist viewpoint, preeclampsia can be considered as a 2-component system in which the 2 parts—the placenta and the mother—fail to connect properly. In theory, this failure could be caused by either component. For example, it is inevitable that CTB differentiation sometimes must go awry. The high frequency of spontaneous abortions that are the results of chromosomal abnormalities are a graphic illustration of the consequences of catastrophic failure of CTB differentiation. But the observation that confined placental mosaicism can be associated with fetal growth restriction<sup>26</sup> is especially relevant to the studies described here as an example of the consequences of partially compromised trophoblast function. Conversely, there is interesting evidence that in certain cases the maternal environment may not permit normal trophoblast invasion. For example, patients with preexisting medical conditions, such as lupus erythematosus and diabetes mellitus, or with increased maternal weight,<sup>27</sup> are prone to developing pregnancy complications, including preeclampsia.<sup>28</sup> Finally, the mother's genotype also may play a role; expression of an angiotensinogen genetic variant has been associated with the predisposition to develop preeclampsia.<sup>29</sup>

An equally interesting area of study is how the faulty link between the placenta and the uterus leads to the fetal and maternal signs of the disease. It is logical that a decrease in maternal blood flow to the placenta could result in fetal growth restriction, a fact that has been confirmed in several animal models.<sup>30</sup> But how this scenario also leads to the dramatic maternal signs of preeclampsia has been far from clear. Recent work has led to important new insights into this enigmatic connection. Specifically, Maynard et al<sup>31</sup> confirmed that placental production of soluble VEGFR-1 (sFlt1) is increased in preeclampsia. Moreover, they showed that increased levels of this receptor circulate in patients' blood, a factor that likely is associated with a parallel decrease in circulating levels of free PlGF. The placental origin of VEGFR-1 was further suggested by showing that it decreases to normal levels after delivery. At a mechanistic level they found that a maneuver that increased circulating levels of soluble VEGFR-1 to pregnant rats induces hypertension, proteinuria, and glomerular endotheliosis, lesions that typically are associated with preeclampsia. Furthermore, it is interesting to note that this observation also may have clinical use. Specifically, as early as the first trimester of pregnancy, increased levels of soluble VEGFR-1 and decreased levels of PlGF predict the subsequent development of preeclampsia.<sup>32,33</sup> To date, these data provide the strongest evidence linking a defect in placentation to the maternal systemic disorder.

In summary, studies of the placenta's role in preeclampsia have reached a very exciting point. We are on the verge of unraveling the series of events that leads from abnormally shallow placentation to this intriguing and potentially catastrophic pregnancy syndrome. Once the connections are clarified, we will be in an excellent position to devise strategies for early diagnosis, and perhaps even treatment, of this condition. In doing so we also will gain vital information about how the normal placenta functions, and about the

critical checkpoints in placental development that govern pregnancy outcome.

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