Expression of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) in conceptus and endometrium during implantation in the rhesus monkey

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The aim of this study was to analyse the expression of transcripts and proteins for vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) in different compartments of the early conceptus at primary implantation sites during lacunar ($n = 6$), early villous ($n = 9$) and villous placenta ($n = 6$) stages of gestation in the rhesus monkey. During the lacunar stage, VEGF expression was observed in the cytotrophoblast cells lining the extraembryonic cavity, but these cells did not express PlGF. With further development, cytotrophoblast cells lining villi, forming columns, and constituting anchoring villi, expressed both VEGF and PlGF during early villous and villous placenta stages. In addition, chorion, amnion and villous stromal cells expressed both VEGF and PlGF proteins and mRNA. During the lacunar stage, all epithelial cells in maternal endometrium generally expressed VEGF, while PlGF expression was observed in the plaque epithelium only. As gestation advanced, the expression of VEGF and PlGF from plaque cells decreased, and in surface and glandular epithelium the expression of VEGF increased, while the expression of PlGF remained unaltered. Decidual stromal cells expressed VEGF and PlGF only at low levels during the lacunar stage, while the expression of both increased during the early villous and the villous placenta stages of implantation. It appears from the present study that the expression of VEGF and PlGF are regulated in a temporal and spatial manner during early stages of implantation and that their concerted actions in placental and maternal compartments play a critical role in the evolving pregnancy in the rhesus monkey.
Key words: cytotrophoblast/implantation/placental growth factor/rhesus monkey/vascular endothelial growth factor

Introduction

Blastocyst implantation in the rhesus monkey is initiated on days 8–9 post-ovulation with the penetration of luminal epithelium by polar syncytiotrophoblast cells. The morphological description of these different cell types and the timing of their appearance in the implantation stage material show close similarities between the non-human species studied (rhesus monkey and baboon) and the human (O'Rahilly, 1973; Hendrickx and Houston, 1976; Luckett, 1978; Enders, 1993). Implantation and placentation involve a series of cellular interactions, which include the formation of mononucleated and multinucleated, motile and non-motile trophoblast cells. Altered patterns of cell proliferation, adhesion and invasion account for the progressive remodelling of tissue at the site of implantation. Only a limited understanding of the endocrine–paracrine relationship of these different cell populations during the process of implantation and early placentation in primates is available (Cross *et al.*, 1994).

An obligatory component of the implantation-associated endometrial reaction in nearly all mammalian species studied is a marked change in the vascular compartment. In addition, neovascularization is an integral event in the process of placental development. Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are strong candidates which may be involved in the process of implantation and placentation (Vuorela *et al.*, 2000). VEGF is a disulphide-linked homodimeric secretory protein, and is a potent angiogenic agent (Connolly *et al.*, 1989). As well as promoting endothelial cell proliferation, it promotes vascular permeability (Keck *et al.*, 1989) and helps to maintain newly formed blood vessels (Alon *et al.*, 1995). VEGF has been suggested as being the principal angiogenic factor secreted by endometrial cells and a stage-specific expression of VEGF isoforms during the menstrual cycle has been observed (Charnock-Jones *et al.*, 1993; Greb *et al.*, 1995; Shifren *et al.*, 1996; Naresh *et al.*, 1999). On the other hand, PlGF is a dimeric factor with a close amino acid sequence homology to VEGF, and it is highly active on endothelial cells in chemotactic, mitogenic and angiogenic assays (Maglione *et al.*, 1991; Ziche *et al.*, 1997). Our knowledge about the involvement of VEGF and PlGF during the early stages of blastocyst implantation in the human is lacking. Ethical and practical constraints do not permit the use of human implantation-stage endometrial and placental samples. Thus, the use of non-human primate species for conducting such studies appears to be warranted. The objective

of the present study was to analyse the expression patterns of these two angiogenic growth factors (VEGF and PlGF) in different compartments of early conceptus at primary implantation sites during the lacunar and villous stages of gestation in the rhesus monkey.

Materials and methods

Animals

Healthy, adult male and female rhesus monkeys housed in the animal quarters of the Primate Research Facility of the All-India Institute of Medical Sciences (AIIMS) were used. The details of animal management are given elsewhere (Ghosh *et al.*, 1996). The experiment was performed with the approval of the Ethics Committee for the Use of Non-Human Primates in Biomedical Research of the AIIMS.

Tissue collection

Female monkeys showing two consecutive cycles of normal length (26–30 days) were placed for mating with proven fertile, male monkeys during days 8–16 of their menstrual cycles. Peripheral blood samples were collected for the estimation of serum concentrations of oestradiol-17β, progesterone, monkey chorionic gonadotrophin (mCG), and vaginal smears were checked for the presence of spermatozoa. The days of ovulation and implantation were detected from serum profiles of oestradiol, progesterone and mCG according to a method described earlier (Ghosh *et al.*, 1997). On estimated days 13–17 ($n = 15$) and 22–24 ($n = 6$) of gestation, monkeys were laparotomized after immobilization with ketamine hydrochloride (12 mg/kg body weight, i.m.; Parke-Davis, Mumbai, India), and subjected to in-situ perfusion fixation with 4% neutral-buffered paraformaldehyde respectively; uteri were then removed by hysterectomy. Each uterine specimen was quickly washed in cold phosphate-buffered saline (PBS) to remove adherent blood and was excised to expose the primary implantation site which was then placed in cold 4% paraformaldehyde fixative for 24 h at 4°C and then further processed through graded dehydration, clearing and embedding in paraffin (Ghosh *et al.*, 1999).

Immunohistochemistry

Immunohistochemical staining was performed with buffered, paraformaldehyde-fixed, deparaffinized tissue sections using the method described previously (Ghosh *et al.*, 1996). Immunohistochemistry (IHC) for VEGF isoform A (R&D Systems, Minneapolis, USA) and PlGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were performed using affinity chromatography-purified antibodies raised in goats against recombinant human antigens. Dilutions of stocks of antibodies for IHC were optimized, based on 3–5 points titrations. According to the suppliers, antibodies for VEGF-A and PlGF had no cross-reactivity with other cytokines. Final visualization was achieved using the ABC peroxidase kits (Vector Laboratories, Burlingame, CA, USA) and freshly prepared diaminobenzidine hydrochloride (Sigma Chemical Co, St Louis, MI, USA) and hydrogen peroxide. Specificity of antibody binding and visualization were assessed by omitting primary antibodies, replacing primary antibodies with unrelated immunoglobulins from the same species and other species, omitting secondary antibodies, and replacing labelled secondary antibody with unrelated labelled immunoglobulins from the same species and other species. Labelled and unlabelled immunoglobulins, non-immune sera and other supplies for IHC were purchased from Vector Laboratories.

In-situ hybridization

The in-situ hybridization protocol used in the present study was based on previously described methods (Angerer and Angerer, 1993; Emson and Gait, 1993), and is briefly described below.

Section preparation

Paraffin sections (5 μm) were dewaxed in xylene and hydrated through graded ethanol to PBS, pH 7.2. Proteinase K (10 μg/ml in PBS; Sigma Chemical Co) digestion was performed at room temperature for 30 min. Following washes in PBS, sections were refixed in freshly made 4% neutral-buffered paraformaldehyde at 4°C, and then washed in PBS. Finally dehydration was carried out using graded ethanol and the sections were air-dried.

Preparation of probes

The probes were generated from polymerase chain reaction (PCR) products amplified from cDNA obtained from term placenta and were cloned into the pCR-Script SK(+) (Stratagene, La Jolla, CA, USA). The orientation and identity of the clones was confirmed using an ABI 373A fluorescent sequencer as described earlier (Clark *et al.*, 1996).

The VEGF probe was a subcloned fragment of a full-length human *VEGF* cDNA. It comprised exons 1–4 and thus would detect all known alternatively spliced variants of VEGF. It spanned the region as described previously (Tischer *et al.*, 1991), from 5′ ATGAAC (amino acid number –26) to TGTGAATGC 3′ (amino acid number 104). This generated a 390 bp probe cloned into the pBluescript II KS vector (Stratagene).

Primers for the amplification of PlGF were 5′GARAARAT-GCCNGCCNGTN ATG 3′ (where R = AG and N = AGCT) beginning at nucleotide 316 as previously described (Maglione *et al.*, 1991) and ending with 5′ CTCCAAGGGGTGGGTTA 3′. This generated a 533 bp probe which contained the 63 bp insert between bases 744 and 745 as previously described (Maglione *et al.*, 1993).

Using linearized templates, single stranded sense and antisense RNA probes were transcribed using a digoxigenin (DIG) RNA labelling kit (Boehringer Mannheim, Mannheim, Germany) and using the protocol supplied by the manufacturer.

Hybridization

Riboprobes (~5 ng/μl) were mixed with 40 μl of hybridization mixture containing deionized formamide (50%, v/v), 50% (w/v) dextran sulphate (20%, v/v), 100× Denhardt's mixture (1%, v/v), Tris (1 mol/l, pH 8.0), sodium chloride, EDTA, sodium phosphate buffer (0.1 mol/l, pH 8.0) and yeast t-RNA (5 mg/l) and were denatured at 80°C. The components for hybridization mixture were obtained from Sigma. The riboprobes were applied to the pretreated and dried sections, and hybridization was carried out for 18 h at 47°C in a sealed humidified chamber equilibrated with 50% formamide and 0.3 mol/l NaCl. Post-hybridization stringency washes were done in 2× sodium chloride/sodium citrate (SSC) and then in 0.1× SSC at 47°C, and subsequently RNAse A (Sigma) digestion was performed as described earlier (Clark *et al.,* 1996). After washing in 2× SSC at 37°C, hybridized probes were detected by using a DIG nucleic acid detection kit (Boehringer Mannheim) following the manufacturer's instructions.

Microscopic assessment

The sections of primary implantation sites after immunostaining and in-situ hybridization were examined using a Leica DMRBE microscope attached with a Leitz DMRD micrographic unit. In the present study three stages of implantation, i.e. lacunar ($n = 6$), early villous ($n = 9$) and villous placenta ($n = 6$) were examined;

Figure 1. A representative micrograph of haematoxylin-stained paraffin section of implantation site obtained on estimated day 15 of gestation. A stage VI embryo is seen with pseudostratified columnar epiblastic plate (asterisk) and amnion (arrowhead). Few cells of endodermal plate are distinguished below the vitelline sac (thin arrow). Primary villi and cell columns of cytotrophoblast cells are seen. Bar = 125 μm.

descriptions of microscopic characteristics of these stages of implantation have been given elsewhere (Ghosh *et al.*, 1999). Figure 1 shows a representation micrograph of haematoxylin-stained paraffin section of a primary implantation site obtained from a pregnant animal on estimated day 15 of gestation. For assessment of staining in cells of embryonic compartments, semi-quantitative subjective scoring was done in blinded manner by three investigators using a 4-scale system: 0 = nil; 1 = weak; 2 = moderate; and 3 = strong (Ghosh *et al.*, 1999). It was assumed that these measurements reflect the concentrations of the experimental probes in different endometrial compartments.

Results

Tables I–III show the median scores with ranges of immuno-histochemical and in-situ hybridization stainings for VEGF and PlGF proteins and transcripts respectively, in different compartments of conceptus and maternal endometrium during the lacunar, early villous and villous placenta stages of implantation. Figures 2 and 3 show representative microphotographs of VEGF and PlGF transcripts and proteins as detected by in-situ hybridization and immunohistochemistry, in different compartments of the conceptus and maternal endometrium during the early stages of blastocyst implantation and placentation.

Expression of VEGF and PlGF in conceptus

During the lacunar stage, VEGF expression was observed in cytotrophoblast cells lining extraembryonic cavity, and weakly in large trophoblast cells within trophoblast plate; however,

there was little expression of PlGF in cytotrophoblast cells lining the extraembryonic cavity (Table I; Figure 2B,F). With development, cytotrophoblast cells lining villi, within cell columns, as well as in anchoring villi, started expressing both PlGF and VEGF (Figures 2C,D,G,H), while extravillous trophoblast cells and intravascular trophoblast cells expressed PlGF and VEGF at a low level (Tables II and III; Figure 3E,F). In addition, chorion, amnion and vilous stromal cells showed both VEGF and PlGF expression. Generally, a high degree of concordance was seen between the profiles of protein and mRNA expression for both VEGF and PlGF in different cell types of conceptus (Figure 2; Tables I, II and III).

Expression of VEGF and PlGF in maternal endometrium

As shown in Table I, during the lacunar stage, all epithelial cells in maternal endometrium generally expressed VEGF (Figure 3A,B), while PlGF expression was observed in the plaque epithelium only (Figure 3C,D). As gestation advanced, the expression of VEGF and PlGF from plaque cells decreased while, in surface and glandular epithelium (Tables II and III), the expression of VEGF increased and the expression of PlGF remained unaltered at a low level. During the lacunar stage, stromal decidual cells expressed VEGF and PlGF at a low to non-detectable level, while the expression of both increased during the early villous and the villous stages of implantation (Tables II and III). Other endometrial cell types did not express either VEGF or PlGF at a notable level (Figure 3).

Discussion

In the present study, we noted that cytotrophoblast cells lining the extraembryonic cavity, villi, columns and anchoring villi express both transcripts and proteins for VEGF in lacunar, early villous and villous stages of implantation and placentation in the rhesus monkey. The cytotrophoblasts also expressed PlGF during villous stages of placentation, but not during the lacunae stage. Syncytiotrophoblast cells and extravillous trophoblasts expressed VEGF and PlGF at low levels. In previous studies, VEGF proteins were seen in cytotrophoblast cells in first trimester human placental samples (Jackson *et al.*, 1994; Ahmed *et al.*, 1995; Clark *et al.*, 1996). However, it is not clear why previous authors (Shiraishi *et al.*, 1996) observed the presence of immunopositive VEGF in syncytiotrophoblast cells at 6–41 weeks of pregnancy, while syncytiotrophoblast cells and extravillous trophoblast cells in first trimester human placental samples exhibited very little VEGF protein in other studies (Jackson *et al.*, 1994; Ahmed *et al.*, 1995; Clark *et al.*, 1996). It has been suggested that such differential localization of VEGF might have been caused by different antibodies recognizing different antigenic epitopes (Shiraishi *et al.*, 1996), as there is evidence that VEGF transcripts may be present in both cytotrophoblast and syncytotrophoblast cells (Sharkey *et al.*, 1993). In the present study, however, transcripts and proteins followed a similar pattern of expression for both VEGF and PlGF in the different cell types.

In an earlier study (Sharkey *et al.*, 1993) using a full length VEGF probe, it was observed that *VEGF* mRNA was expressed
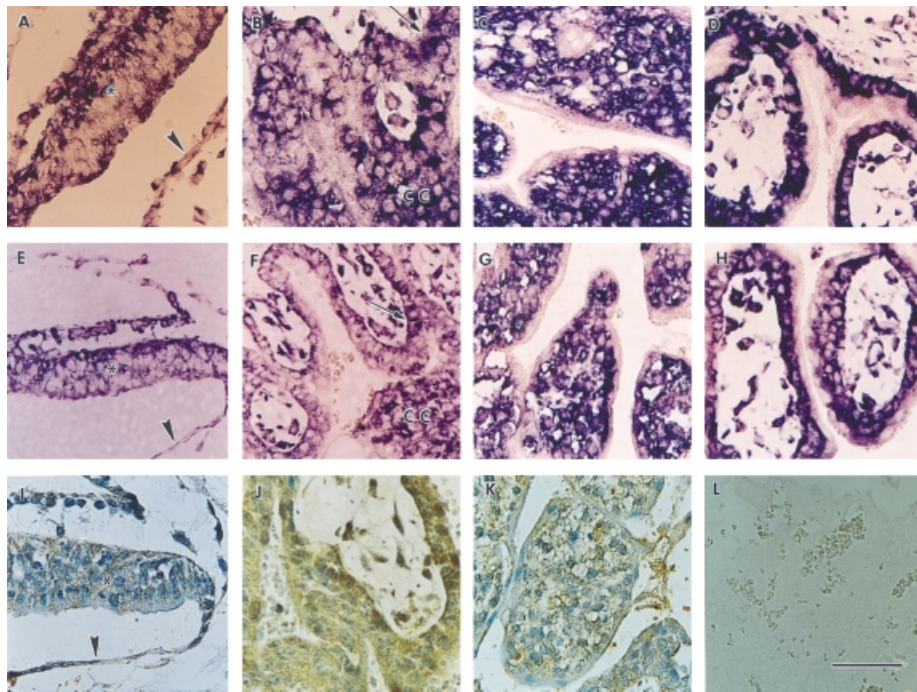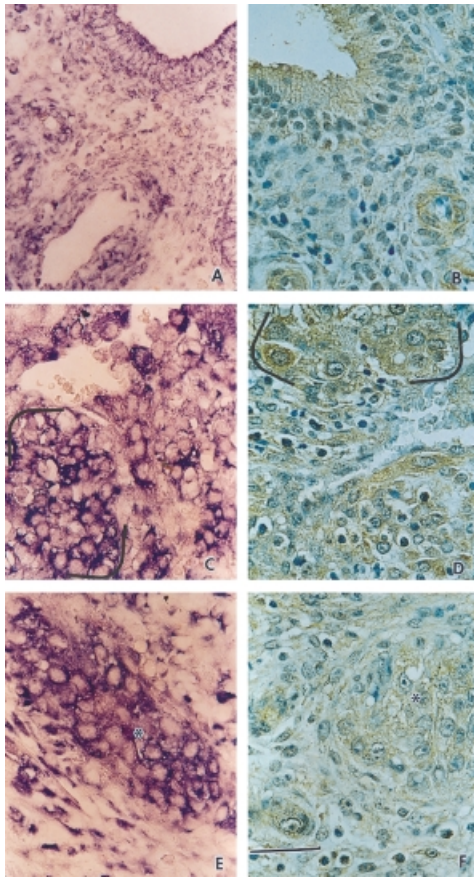
Figure 2. In-situ hybridization (**A–H**) and immunohistochemistry (**I–K**) for vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) in primary implantation sites. (**A–D**) *VEGF* mRNA and (**E–H**) *PlGF* mRNA expression is seen in pseudostratified columnar cells of embryonic disc (asterisk) and (**A, E**) in cells of developing amnion (arrowhead) in stage VI embryo; (**B, F**) in cytotrophoblast cells lining extraembryonic cavity (arrow) and developing cell columns (CC); (**C, G**) in anchoring villi; and (**D, H**) in the lining of floating villi. (**L**) In-situ hybridization with the VEGF sense probe did not show any detectable stain in the villous implantation site. (**I**) Immunopositive staining for VEGF protein was detected in epiblast cells (asterisk) and (**J**) primary villi; and (**K**) staining for PlGF in anchoring villi. (**A, I, J**, and **K**) bar = 30 µm; (**B–H**, and **L**) bar = 60 µm.

in first trimester human villous cytotrophoblasts, as well as in syncytiotrophoblast cells and extravillous trophoblast cells. Using a probe directed against exons 1–4 of the *VEGF* gene, and having a very low homology with other members of the VEGF family, it was observed (Clark *et al.*, 1998) that villous trophoblast cells of first trimester human placenta did not express *VEGF* mRNA. Another study (Vuorela *et al.*, 1997) also showed that there was no *VEGF* mRNA and protein signal in villous trophoblasts in human term placenta. Thus, it is possible that the full length probe used in the previous studies cross-reacted with PlGF in villous trophoblasts, because PlGF shares close amino acid homology to VEGF. This assumption is substantiated by the observation that *PlGF* mRNA was expressed by villous cytotrophoblast cells in both the present study and in previous reports (Shore *et al.*, 1997; Vuorela *et al.*, 1997; Clark *et al.*, 1998).

In the present study, however, we have used a probe directed against exons 1–4 of VEGF and thus, it is unlikely that it cross-reacted with PlGF transcripts. Moreover, there was very little *PlGF* mRNA expression during the lacuna stage, when VEGF was expressed to a moderate level in cytotrophoblast cells. From both this and previous studies it appears that, during the initial stages of blastocyst implantation, trophoblast cells express VEGF, and with advancement in the process of placentation, VEGF expression gradually decreases, while PlGF expression increases. The biological regulation and significance of such switch overs are not known. However, it is known that hypoxia up-

regulates VEGF and down-regulates PlGF (Ahmed and Kilby, 1997). Thus, it is possible that hypoxic stress at the time of implantation may induce VEGF, which may directly or indirectly influence endometrial perfusion, proliferation of trophoblast cells, trophoblast invasion, platelet aggregation and villus formation (Genbacev *et al.*, 1996; Ahmed *et al.,* 1997; Ahmed and Kilby, 1997; Wheeler-Jones *et al.*, 1997; Murohara *et al.*, 1998). As a result, the oxygen tension gradients between maternal decidua and placental villi decrease, and PlGF may be induced which, together with VEGF, influences villus formation, vasculogenesis and trophoblast cell invasion. It is, therefore, possible that stage-dependent induction of VEGF and PlGF may be critical for the establishment of placental sufficiency and, hence, embryonic development despite the fact that PlGF shares 53% sequence homology with VEGF (Krebs *et al.*, 1996; Macara *et al.*, 1996). Differential expression of VEGF and PlGF in trophoblast cells in a stage-specific manner may be important, because these factors may mutually regulate their effects on target cells (DiSalvo *et al.*, 1995; Cao *et al.*, 1996; Murohara *et al.*, 1998). Whether such temporal and spatial patterns of expression of VEGF and PlGF are associated with that of different receptor moieties for VEGF, i.e. kinase domain-containing region (KDR) and *fms*-like tyrosine kinase (Flt) 1, remains to be investigated. PlGF secreted by placental tissue may regulate the growth and function of trophoblast cells and endometrium through both the autocrine and paracrine routes via Flt-1 receptors

Figure 3. In-situ hybridization and immunohistochemistry for (**A**, **B**) vascular endothelial growth factor (VEGF) and (**C–F**) placental growth factor (PlGF) in maternal endometrium associated with primary implantation sites. (**A**) *VEGF* mRNA and (**B**) protein expression in glandular epithelium and in vascular compartment, and (**C**, **E**) *PlGF* mRNA transcript and (**D**, **F**) protein expression in (brackets; **C**, **D**) epithelial plaque cells and (asterisk; **E**, **F**) intravascular trophoblast cells. (**A**) Bar = 60 µm; (**B–F**) bar = 30 µm.

(Athanassiadas and Lala, 1998; Desai *et al.*, 1999). It is to be noted that the PlGF homodimer binds only to Flt-1, but not KDR, while both types of receptor bind to VEGF (Park *et al.*, 1994; Terman *et al.*, 1994; Cao *et al.*, 1996).

Similar to previous observations (Sharkey *et al.*, 1993; Ahmed *et al.*, 1995; Shiraishi *et al.*, 1996; Clark *et al.*, 1998), VEGF and PlGF were found to be expressed in chorion, amnion and villous stromal cells to a variable extent in the present study. The physiological significance of this expression is not known, but it is probable that VEGF and PlGF produced by fetal membranes and villous stromal cells may act locally on placental blood vessels (Ahmed *et al.*, 1995).

Although there is no information available in the literature regarding the expression of VEGF and PlGF in maternal endometrium during the early stages of placentation in the human, our observations agree well with the data obtained from first trimester human maternal decidua (Sharkey *et al.*, 1993; Clark *et al.*, 1996, 1998). The expression of VEGF by maternal epithelial cells of luminal surface, glands and plaque acini during early stages of implantation appears interesting. It has been demonstrated previously that VEGF is secreted by

Table I. Vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) in fetal and maternal compartments during the lacunar stage. Values are given as median score with range in parentheses

Cell type	Score			
	VEGF		PlGF	
	IHC	ISH	IHC	ISH
Fetal compartment				
Chorion	2 (1–2)	1 (1–2)	2 (1–3)	1 (0–2)
Embryonic disc				
Endoderm	0 (0–1)	0 (0–1)	0 (0–1)	1 (0–1)
Ectoderm	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Amnion	1 (0–2)	0 (0–1)	0 (0–2)	0 (0–1)
Cytotrophoblasts lining embryonic cavity	2 (1–3)	1 (1–3)	0 (0–1)	0 (0–2)
Syncytiotrophoblasts lining lacunae	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–1)
Large trophoblast cells in trophoblast plate	1 (1–2)	1 (1–2)	2 (1–2)	1 (1–2)
Maternal compartment				
Surface epithelium	2 (1–2)	1 (1–2)	0 (0–1)	0 (0–1)
Glandular epithelium	1 (1–2)	1 (0–2)	0 (0–1)	0 (0–1)
Plaque cells	2 (1–2)	1 (1–2)	1 (0–2)	1 (0–3)
Decidual cell	1 (0–2)	0 (0–1)	1 (0–2)	1 (0–3)
Fibroblast cells	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–1)
Endothelial cells	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–1)
Vascular smooth cells	2 (0–2)	0 (0–1)	1 (0–2)	0 (0–1)

IHC = immunohistochemistry; ISH = in-situ hybridization.

Table II. Vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) in fetal and maternal compartments during the early villous stage. Values are given as median score with range in parentheses

Cell type	Score			
	VEGF		PlGF	
	IHC	ISH	IHC	ISH
Fetal compartment				
Chorion	2 (1–3)	1 (0–1)	2 (1–3)	1 (0–1)
Embryonic disc				
Endoderm	1 (0–1)	1 (0–1)	0 (0–1)	1 (0–1)
Ectoderm	0 (0–1)	1 (0–1)	0 (0–2)	1 (0–2)
Amnion	1 (0–2)	0 (0–1)	1 (0–2)	0 (0–1)
Cytotrophoblasts lining villi	2 (1–3)	2 (1–3)	2 (1–3)	3 (1–3)
Syncytiotrophoblasts lining villi	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Villous stromal cells	3 (2–3)	2 (1–3)	3 (1–3)	3 (1–3)
Non-polarized cytotrophoblast cells forming columns	3 (1–3)	2 (1–3)	3 (2–3)	2 (1–3)
Syncytiotrophoblast cells lining cytotrophoblast cell column	1 (1–2)	0 (0–1)	0 (0–1)	0 (0–1)
Trophoblast cells in dilated venules	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)
Maternal compartment				
Surface epithelium	2 (2–3)	1 (0–3)	1 (1–3)	1 (0–2)
Glandular epithelium	2 (1–3)	1 (0–2)	1 (1–2)	1 (0–1)
Plaque cells	1 (0–2)	1 (1–2)	1 (1–2)	2 (1–3)
Decidual cell	2 (1–3)	2 (1–2)	2 (1–3)	2 (1–2)
Fibroblast cells	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Endothelial cells	0 (0–1)	1 (1–2)	0 (0–1)	1 (0–2)
Vascular smooth cells	1 (1–3)	0 (0–1)	0 (0–2)	0 (0–1)

IHC = immunohistochemistry; ISH = in-situ hybridization.

polarized human endometrial epithelial cells both *in vivo* and *in vitro* (Greb *et al.*, 1995; Shifren *et al.*, 1996; Torry *et al.*, 1996), and it has been suggested that its vectorial secretion

Table III. Vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) in fetal and maternal compartments during the villous stage of placentation. Values are given as median score with range in parentheses

Cell type	Score			
	VEGF		PlGF	
	IHC	ISH	IHC	ISH
Fetal compartment				
Chorion	2 (1–3)	1 (1–2)	3 (1–3)	2 (1–3)
Cytotrophoblast cells lining villi	1 (1–3)	1 (0–2)	2 (2–3)	2 (1–3)
Syncytiotrophoblasts lining villi	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Villous stromal cells	2 (1–3)	3 (2–3)	1 (1–3)	3 (2–3)
Developing blood vessels	1 (1–3)	0 (0–1)	2 (1–3)	0 (0–2)
Non-polarized cytotrophoblast cells in anchoring villi	1 (0–2)	2 (1–3)	2 (1–3)	3 (2–3)
Syncytiotrophoblasts lining anchoring villi	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Cytotrophoblast shell	0 (0–2)	0 (0–2)	0 (0–1)	0 (0–1)
Maternal compartment				
Surface epithelium	3 (2–4)	2 (2–3)	1 (1–3)	1 (0–2)
Glandular epithelium	2 (1–3)	1 (1–2)	1 (0–2)	1 (0–2)
Decidual cell	2 (2–3)	2 (1–3)	2 (1–3)	2 (1–3)
Fibroblast cells	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Endothelial cells	0 (0–1)	2 (0–3)	0 (0–1)	0 (0–2)
Vascular smooth cells	1 (0–2)	0 (0–1)	0 (0–1)	0 (0–1)

IHC = immunohistochemistry; ISH = in-situ hybridization.

may co-ordinate embryo development with the implantation process (Hornung *et al.*, 1998).

Evolution of plaque epithelium as a maternal endometrial response to the implanting blastocyst is seen in monkeys. The physiological significance of the emergence of plaque cells is not known, although ultrastructurally they appear to possess a secretory function which may be linked with the development of maternal vasculature, and eventual placental development as the expanding placental lacunae communicate with the enlarged vascular bed in the maternal endometrium (Enders *et al.,* 1985). Our observation that plaque epithelial cells express both VEGF and PlGF indirectly support this speculation about the function of plaque cells. In the same manner, production and secretion of VEGF and PlGF by stromal decidual cells may influence vascular responses during implantation, because of their close proximity to blood vessels (Rogers *et al.*, 1992). It is to be noted in this connection that, in the rhesus monkey, epithelial plaque cell differentiation occurs as an immediate response of maternal endometrium to blastocyst implantation which predictably is initiated on days 7–8 after fertilization, while typical stromal decidual cell transformation is initiated around day 15 of gestation. On the other hand, implantation associated endometrial vascular changes characterized by enhanced permeability, decreased vascular impedence, vasodilatation and increased endothelial cell proliferation are seen by days 10–13 of gestation (see Enders *et al.*, 1985 for details). Thus, it is possible that plaque epithelium and stromal decidual cells regulate the maternal vascular responses in a time-synchronized manner, and thereby influence placental development through both VEGF and PlGF, in a discrete manner.

Collectively, it appears that VEGF and PlGF are highly regulated growth factors during the early stages of implantation in the primate and that their concerted actions in fetal placenta and maternal endometrium play a critical role in mediating an evolving symbiotic interaction between these two compartments in the monkey.

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