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Ovarian Steroid Regulation of Vascular Endothelial Growth Factor in the Human Endometrium: Implications for Angiogenesis during the Menstrual Cycle and in the Pathogenesis of Endometriosis*

JAN L. SHIFREN†, JENNIFER F. TSENG‡, CHARLES J. ZALOUDEK, ISABELLE P. RYAN, Y. GLORIA MENG, NAPOLEONE FERRARA, ROBERT B. JAFFE, AND ROBERT N. TAYLOR

Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences (J.L.S., J.F.T., I.P.R., R.B.J., R.N.T.), and the Department of Pathology (C.J.Z.), University of California, San Francisco, California 94143; and the Departments of BioAnalytical Technology (Y.G.M.) and Cardiovascular Research (N.F.), Genentech, Inc., South San Francisco, California 94080

ABSTRACT

The human endometrium undergoes a complex process of vascular and glandular proliferation, differentiation, and regeneration with each menstrual cycle in preparation for implantation. Vascular endothelial growth factor (VEGF) is an endothelial cell-specific angiogenic protein that appears to play an important role in both physiological and pathological neovascularization. To investigate whether VEGF may regulate human endometrial angiogenesis, we examined VEGF messenger ribonucleic acid (mRNA) and protein throughout the menstrual cycle and studied the regulation of VEGF by reproductive steroids in isolated human endometrial cells. By ribonuclease protection analysis, VEGF mRNA increased relative to early proliferative phase expression by 1.6-, 2.0-, and 3.6-fold in midproliferative, late proliferative, and secretory endometrium, respectively. In histological sections, VEGF mRNA and protein were localized focally in glandular epithelial cells and more diffusely in surrounding stroma, with greatest VEGF expression in secretory endometrium. Consistent with these *in vivo* results, the treatment of isolated human endometrial cells with estradiol (E_2), medroxyprogesterone acetate (MPA), or E_2 plus MPA significantly increased VEGF mRNA expression over the control value by 3.1-, 2.8-, and 4.7-fold, respectively. The VEGF response to E_2 was rapid, with steady state levels of VEGF mRNA reaching 85% maximum 1 h after the addition of steroid. E_2 also caused a 46% increase in secreted VEGF protein, and the combination of E_2 and MPA caused an 18% increase. VEGF expression in endometriosis, an angiogenesis-dependent, estrogen-sensitive disease was similar to that seen in eutopic endometrium. Peritoneal fluid concentrations of VEGF were significantly higher in women with moderate to severe endometriosis than in women with minimal to mild endometriosis or no disease. VEGF, therefore, may be important in both physiological and pathological angiogenesis of human endometrium, as it is an estrogen-responsive angiogenic factor that varies throughout the menstrual cycle and is elevated in women with endometriosis. (*J Clin Endocrinol Metab* **81:** 3112–3118, 1996)

THE RAPID growth of the endometrium in preparation for implantation requires dynamic vascular remodeling. Although human endometrium has angiogenic activity (1), the factors regulating endometrial angiogenesis have not been extensively characterized. During the menstrual cycle, there is a gradual increase in the length, branching, and coiling of spiral arteries (2). These end arterioles supply the functional layer of the endometrium and are highly sensitive to ovarian steroids (1). During the late proliferative phase and throughout the secretory phase, a complex subepithelial capillary plexus develops.

Ovarian steroid hormones direct differentiation of the endometrium during the menstrual cycle. However, although uterine cells are very sensitive to estrogen *in vivo*, they fail to respond fully to physiological doses of estrogen *in vitro*. Polypeptide growth factors regulate endometrial proliferation and differentiation and may mediate the effects of estrogen and progesterone on the endometrium in autocrine and/or paracrine manners (3, 4). Although the effects of growth factors and ovarian steroids on epithelial and stromal development have been analyzed in several studies, little is known about angiogenic factors that may affect the endometrial vasculature throughout the menstrual cycle. Acidic and basic fibroblast growth factor stimulate vascular endothelial cell growth *in vitro* and angiogenesis *in vivo* (5, 6) and are present in human endometrium. However, these angiogenic factors are unlikely to be principal regulators of cyclic endometrial neovascularization, as their expression does not change throughout the menstrual cycle and actually increases in atrophic menopausal endometrium (7, 8).

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Address all correspondence and requests for reprints to: Robert N. Taylor, M.D., Ph.D., Reproductive Endocrinology Center, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California 94143-0132.

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Vascular endothelial growth factor (VEGF) is a heparin-binding dimeric glycoprotein with growth-promoting activity specific for vascular endothelial cells. It has a typical signal sequence permitting active secretion from intact cells (9). In addition to being a mitogen for endothelial cells, VEGF increases vascular permeability (10, 11) and modulates the expression of several proteolytic enzymes involved in angiogenesis (12, 13). Four molecular species of VEGF have been identified ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$) that are generated by alternative mRNA splicing of a single gene (14). A fifth splice variant, $VEGF_{145}$, has been detected recently in endometrium (15). Several studies suggest that VEGF may be important in angiogenesis in the female reproductive tract (16).

Endometriosis, a gynecological disorder associated with dysmenorrhea, pelvic pain, and infertility, affects 10% of women of reproductive age in the United States (17). Ovarian steroids modulate the development and progression of endometriosis, and hormonal manipulation forms the basis of medical treatment (18). Endometriosis probably arises from the peritoneal seeding of viable endometrial cells during retrograde menstruation (19). As this event occurs in ~90% of cycling women (20, 21), additional factors must be evoked to explain why the disease occurs in some women and not in others (22). Angiogenesis is likely to be involved in the pathogenesis of endometriosis, as the successful implantation and growth of endometrial tissue require a new vascular supply. Endometriotic implants tend to be surrounded by increased vascularity, and extrapelvic endometriosis is found in well vascularized sites, such as lung, skin, and muscle (23). The peritoneal fluid from women with endometriosis has increased angiogenic activity compared to that from women without this disorder (24, 25). Variation in endometrial angiogenic activity among women may be one determinant of successful implantation and progression of endometriosis.

Successful growth of eutopic and ectopic endometrium requires the synchronous proliferation of epithelial, stromal, and vascular endothelial cells. As local growth factors are known to mediate the effects of estrogen and progesterone on glands and stroma, the parallel regulation of VEGF by ovarian steroids would serve to coordinate cyclic endometrial development. Therefore, we examined the ovarian steroid regulation of VEGF in the human endometrium as well as assessed a possible role for this growth factor in the pathogenesis of endometriosis.

Materials and Methods

Sources of eutopic endometrium, endometriosis, and peritoneal fluid

Peritoneal fluid samples and biopsies of endometrium and endometriosis were obtained from consenting patients as we have reported previously (22, 25). Peritoneal fluid was aspirated immediately on entering the peritoneal cavity, cells were removed by centrifugation, and 0.1 μg aprotinin/mL was added to the supernatant. Fluid samples were concentrated in Centricon 10 columns (Amicon, Beverly, MA). Hemorrhagic fluids were excluded. The pelvis was staged according to a modification of the revised American Fertility Society (AFS) system (25, 26). Control subjects were women without pelvic pathology. All study protocols were approved by the committee on human research at University of California-San Francisco.

Human endometrial cell cultures

Primary endometrial cell cultures were prepared from biopsies as we have described previously (22). Glandular epithelial cells were separated from stromal cells and debris by filtration through narrow gauge sieves. Stromal cells were subcultured to eliminate contamination by macrophages or other leukocytes, and experiments were performed at passage 1. Extensive characterization of cell cultures prepared using this protocol confirmed that they were more than 95% pure and retained functional markers of their endometrial origin *in vitro* (22).

Steroid treatment of endometrial cell cultures

Cells were cultured in serum-free medium before the addition of 10 nmol/L estradiol (E_2) and/or 100 nmol/L medroxyprogesterone acetate (MPA) (15, 27). MPA was used in place of natural progesterone because it is more slowly metabolized in tissue culture.

Extraction and purification of RNA from tissues and cells

Total RNA was extracted from endometrial biopsies and cell cultures using the method of Chomczynski and Sacchi (28, 29).

Ribonuclease protection analysis

For ribonuclease (RNase) protection analysis, a 381-base ^{32}P-labeled VEGF complementary RNA (cRNA) probe, corresponding to nucleotides 937-1238 of human VEGF, was synthesized by *in vitro* transcription and hybridized with 10 μg total RNA, as we have described previously (29). The abundance of β_2-microglobulin was assayed simultaneously as a standard. Data were analyzed as ratios of the density of the hybridization signals of VEGF to β_2-microglobulin, as determined by computer-assisted densitometry (Scan Analysis, Biosoft, Ferguson, MO).

Northern blot analysis

Total RNA (10 μg) was subjected to electrophoresis and blotted by capillary transfer onto a nylon membrane (29). The membrane was hybridized with a ^{32}P-labeled VEGF complementary DNA (cDNA) probe synthesized by random primer extension. The template for the cDNA probe is a 921-bp fragment of VEGF (protein-coding region), corresponding to nucleotides 335-1256 (9). The integrity and relative amount of RNA loaded into each lane were confirmed using a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ^{32}P-labeled cDNA probe as a constitutively expressed marker. Data were analyzed as ratios of the density of the hybridization signals of VEGF to GAPDH, as determined by computer-assisted densitometry (Scan Analysis, Biosoft, Ferguson, MO).

Immunohistochemistry

VEGF protein was localized in serial sections (10 μm) of paraffin-embedded endometrial and endometriosis biopsies using modifications of an avidin-biotin-peroxidase method (Vector Laboratories, Burlingame, CA), as we have previously reported (29). Sections were incubated overnight with a rabbit polyclonal anti-VEGF antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a concentration of 10 μg/mL at 4 C in a humidified chamber. Normal rabbit serum at the same concentration was used as the control. The sections were lightly counterstained with hematoxylin.

In situ hybridization

VEGF mRNA was localized in serial sections (10 μm) of paraffin-embedded endometrial and endometriosis biopsies by *in situ* hybridization using fluoresceinated sense and antisense riboprobes (Amersham, Aylesbury, UK). The riboprobe corresponds to nucleotides 145–284 of human VEGF cDNA (30) and recognizes all four reported human transcript isoforms. After hydration, sections were treated with proteinase K (100 mg/mL) for 13 min at 37 C, washed with glycine (2 mg/mL) in phosphate-buffered saline (PBS) for 5 min, incubated in 20% acetic acid at 4 C for 15 s, washed again in PBS, and then air dried. Hybridization was performed at 50 C overnight with an approximate probe concentration of 400 ng/mL. To reduce background, sections were

treated with RNase A (10 μg/mL) in 2 × SSC (standard saline citrate) at 37 C for 20 min and rinsed in 2 × SSC at 25 C for 5 min. Sections were washed in Tris-buffered saline (TBS) at 25 C for 5 min, incubated in 0.5% blocking solution at 25 C for 1 h, rinsed in TBS at 25 C for 1 min, and reacted with an antifluorescein antibody conjugated to alkaline phosphatase diluted 1:1000 in 0.5% BSA fraction V in TBS at 25 C for 1 h. Sections were then washed three times in TBS at 25 C for 5 min each time and in detection buffer at 25 C for 5 min. Nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate were applied, and sections were incubated in the dark for 6–9 h at room temperature. Hybridization signal was represented by the deposition of a blue/purple precipitate visualized by light microscopy. Sections were not counterstained.

VEGF enzyme-linked immunosorbent assay (ELISA)

ELISA plates were coated with 2.5 μg/mL monoclonal antibody to VEGF (mAb 3.5F8) in 50 mmol/L carbonate buffer, pH 9.6, at 4 C overnight and blocked with 0.5% BSA in PBS. All monoclonal antibodies were prepared and specificity was characterized as previously described (31). Standards (0.03–2 ng/mL recombinant $VEGF_{165}$) and 3-fold serially diluted samples (initial dilution, 1:5 of the concentrated samples) in PBS containing 0.5% BSA, 0.05% polysorbate 20, 0.25% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 0.2% bovine gamma-globulins (Sigma Chemical Co., St. Louis, MO), 5 mmol/L ethylenediamine tetraacetate, and additional 0.35 NaCl were incubated on the plate for 2 h. Bound VEGF was detected using biotinylated monoclonal antibody to VEGF (mAb 4.6.1), followed by streptavidin peroxidase (Sigma) and 3,3′,5,5′-tetramethyl benzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD) as the substrate. Absorbance was read at 450 nm on a Vmax plate reader (Molecular Devices, Menlo Park, CA). The standard curve was fitted using a four-parameter nonlinear regression curve-fitting program (developed at Genentech, South San Francisco, CA). Data points that fell in the linear range of the standard curve were used for calculating the VEGF concentration in samples. The assay was linear for VEGF in conditioned medium and peritoneal fluid and was sensitive to 0.03 ng/mL.

Statistical analysis

All experiments were repeated a minimum of three times. Normally distributed data were analyzed by paired *t* test or ANOVA, with Scheffe's F test correction for multiple comparisons. Nonparametric data were analyzed by the Kruskal-Wallis statistic. Linear regression analysis was performed to determine the degree of correlation between variables (32). Significant differences were accepted when two-tailed analyses yielded $P < 0.05$.

Results

VEGF expression during the menstrual cycle

Steady state levels of VEGF mRNA in human endometrium from various phases of the menstrual cycle were analyzed by RNase protection analysis (Fig. 1). To control for variation in mRNA loading, β_2-microglobulin mRNA levels were determined concurrently, and data were analyzed as ratios of the density of the hybridization signals of VEGF to β_2-microglobulin. Relative to VEGF expression in endometrium from the early proliferative phase, VEGF mRNA increased in the midproliferative, late proliferative, and secretory phases by 1.6-, 2.0-, and 3.6-fold, respectively. This increase was observed after accounting for the apparent increase in mRNA loading across the menstrual cycle, demonstrated by increasing levels of β_2-microglobulin mRNA. Several smaller bands, observed below the protected fragment at 302 bp, most likely represented VEGF mRNA splice variants.

To confirm that VEGF expression increased during the late proliferative and secretory phases of the menstrual cycle and to localize VEGF protein and mRNA to specific cell types,

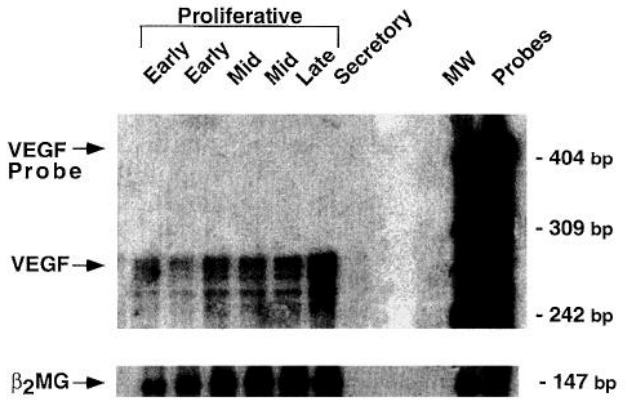

FIG. 1. The relative abundance of VEGF mRNA in human endometrium from early, mid-, and late proliferative and secretory phases of the menstrual cycle demonstrated by RNase protection analysis. The amount of total RNA assayed was confirmed by concurrent hybridization with a β_2-microglobulin (β_2 MG) probe. The full-length probe for VEGF (381 bp) and the expected protected fragments for VEGF (302 bp) and β_2-microglobulin (144 bp) are noted by *arrows*. Endometrial VEGF expression increased relative to early proliferative phase expression approximately 1.6-, 2.0-, and 3.6-fold in midproliferative, late proliferative, and secretory endometrium, respectively.

immunohistochemistry and *in situ* hybridization were performed (Fig. 2, A–F). Fixed sections of endometrium were analyzed for VEGF protein and mRNA. Throughout the menstrual cycle, VEGF was localized in the cytoplasm and was focally concentrated in endometrial glands, with more diffuse staining in surrounding stroma. In proliferative phase endometrium, VEGF protein (Fig. 2A) and mRNA (Fig. 2B) were seen primarily in glandular epithelial cells. Greater signal intensity for VEGF protein (Fig. 2C) and mRNA (Fig. 2D) was observed in secretory endometrium. Serial control sections of endometrium incubated with normal rabbit serum (Fig. 2E) and sense VEGF cRNA (Fig. 2F) showed no specific staining.

Regulation of VEGF by ovarian steroids

To determine whether ovarian steroids directly regulate VEGF expression, isolated endometrial stromal cells were grown to confluence and treated for 24 h with E_2 (10 nmol/L) and/or MPA (100 nmol/L; Fig. 3). Total RNA was analyzed by Northern blot for VEGF mRNA, using GAPDH mRNA to control for RNA loading. Data from seven independent experiments were analyzed as ratios of the density of the hybridization signals of VEGF to GAPDH. VEGF transcripts of approximately 4.4 and 4.2 kilobases (kb) were detected. The addition of E_2, MPA, and E_2 plus MPA significantly increased VEGF expression over control conditions ($P = 0.01$). The increase, expressed as the mean ± SEM, was 3.1 ± 1.8 for E_2, 2.8 ± 2.1 for MPA, and 4.7 ± 3.8 after the addition of both steroids.

Isolated human endometrial stromal cells were treated with E_2 (10 nmol/L) for various lengths of time to determine the rapidity of action on VEGF expression. Although maximal steady state VEGF mRNA levels were seen at 24 h, an increase in VEGF mRNA to 85% of the peak value was seen as early as 1 h after the addition of E_2 (Fig. 4).

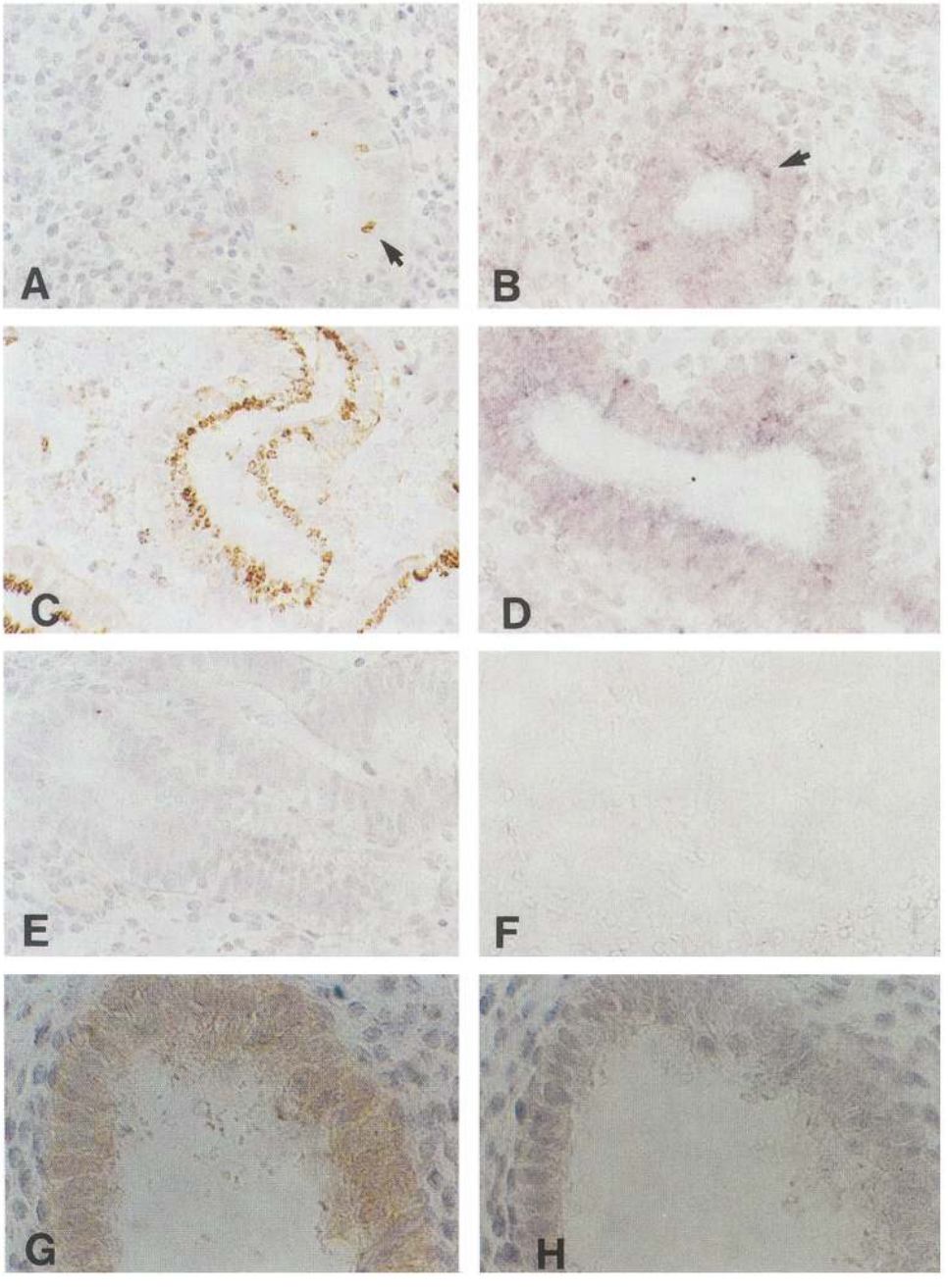

FIG. 2. Cellular localization of VEGF protein and mRNA in human endometrium and endometriosis demonstrated by immunohistochemistry and *in situ* hybridization. VEGF protein (A) and mRNA (B) were detected in proliferative endometrium (magnification, ×300). VEGF was localized focally in the cytoplasm of glandular epithelial cells (*arrows*) and more diffusely in surrounding stroma. Greater signal intensity for VEGF protein (C) and mRNA (D) was observed in secretory endometrium (×300). Serial control sections of secretory endometrium incubated with normal rabbit serum (E) and sense VEGF cRNA (F) showed no specific staining. VEGF protein was present in endometriotic tissue and distributed in a manner similar to that observed in normal endometrium (G). Staining was absent in a serial control section of endometriosis incubated with normal rabbit serum (H; magnification, ×480).

Conditioned media were collected after 24 h and assayed for secreted VEGF by ELISA. VEGF secreted from isolated human endometrial stromal cells was increased 46% after the addition of E_2 and 18% after the addition of E_2 and MPA combined ($P < 0.04$; Fig. 5). VEGF concentrations in conditioned medium ranged from 0.03–0.16 ng/mL.

VEGF expression in endometriosis

Peritoneal implants from women with endometriosis were analyzed for the presence of VEGF by immunohistochemistry. Similar to its localization in eutopic endometrium, VEGF was expressed focally in glandular epithelial cells and diffusely in surrounding stroma of endometriosis tissues (Fig. 2G). No specific staining was seen in serial sections incubated with normal rabbit serum (Fig. 2H).

Peritoneal fluid from women with endometriosis and from those without the disease was collected and assayed for secreted VEGF by ELISA. Women with moderate to severe endometriosis (modified AFS stages III and IV) had significantly higher concentrations of peritoneal fluid VEGF than women without disease ($P < 0.05$) or those with minimal to mild disease (modified AFS stages I and II; $P < 0.05$; Fig. 6). There was a positive correlation ($r = 0.54$) between the severity of endometriosis and the concentration of VEGF in peritoneal fluid ($P = 0.01$).

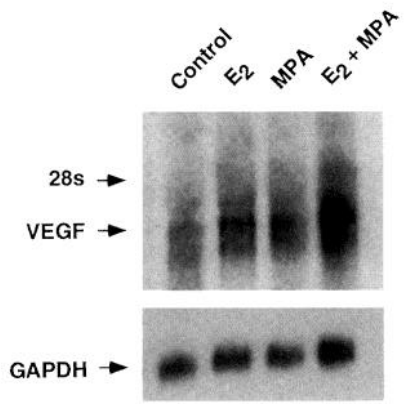

FIG. 3. Northern analysis demonstrating the regulation of steady state VEGF mRNA levels in cultured human endometrial stromal cells by E_2 (10 nmol/L) and MPA (100 nmol/L). VEGF transcripts of approximately 4.4 and 4.2 kb were detected. The position of 28S ribosomal RNA on the original gel is marked. The integrity and amount of total RNA loaded were confirmed by subsequent hybridization of the blot with a GAPDH probe (1.2 kb).

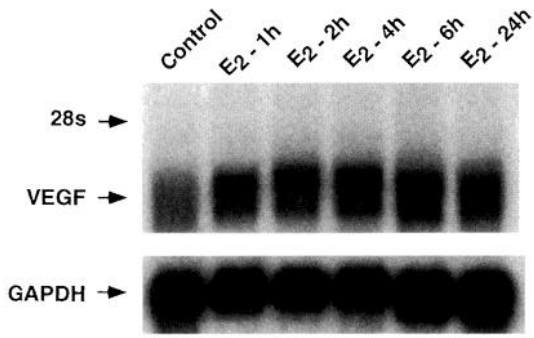

FIG. 4. Northern analysis demonstrating the time required for E_2 (10 nmol/L) to increase VEGF steady state mRNA levels in cultured human endometrial stromal cells. VEGF mRNA was increased to 85% of the maximum value seen at 24 h as early as 1 h after the addition of E_2.

Discussion

VEGF expression during the menstrual cycle

Our studies describe for the first time the cellular localization and relative abundance of both VEGF mRNA and protein in the cycling human endometrium. Shweiki *et al.* (33) first described cyclic variation in VEGF mRNA in rodent endometrium and suggested that VEGF expression may be hormonally mediated. In these studies, VEGF was localized to epithelium and then to stroma under the influence of estrogen and progesterone, respectively (33). In the first study of VEGF in human endometrium, Charnock-Jones *et al.* (15), examining the localization of VEGF transcripts only, observed the opposite pattern. VEGF mRNA was observed mainly in stroma during the proliferative phase and in glandular epithelium during the secretory phase. In a recent study of endometrial VEGF expression in the nonhuman primate, Greb *et al.* (34) observed VEGF protein to be localized primarily to the cytoplasm of glandular epithelial cells, accompanied by diffuse stromal staining. The intensity of VEGF staining increased in the secretory phase throughout the endometrium.

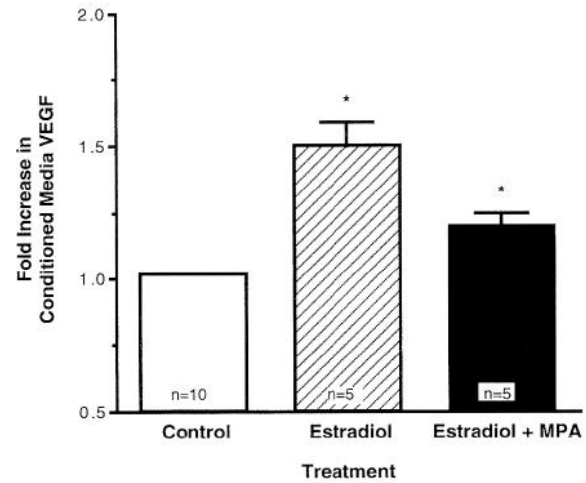

FIG. 5. ELISA analysis of VEGF protein concentration secreted from cultured human endometrial stromal cells treated with E_2 (10 nmol/L) and E_2 plus MPA (100 nmol/L) for 24 h. Conditioned medium VEGF increased 46% after the addition of E_2 and 18% after the addition of E_2 plus MPA (*, $P < 0.04$).

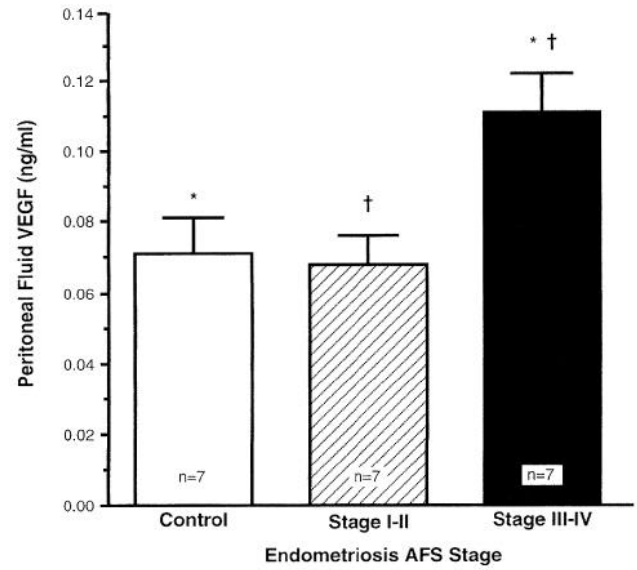

FIG. 6. ELISA analysis of VEGF protein concentrations in the peritoneal fluid of women with and without endometriosis. Peritoneal fluid VEGF levels were significantly higher in women with moderate to severe endometriosis (AFS stages III–IV) compared to women with minimal to mild endometriosis (AFS stages I–II; †, $P < 0.05$) or to women with no evidence of disease (*, $P < 0.05$).

The pattern of expression that we observed for VEGF mRNA and protein in human endometrium is similar to the distribution of VEGF protein described by Greb *et al.* (34) in the nonhuman primate. In the human, VEGF mRNA and protein were localized focally in glandular epithelial cells, with more diffuse localization in stromal cells throughout the cycle. Although VEGF localized to endometrial stroma could in part represent macrophage production, as macrophages reside in the endometrium and are known to express VEGF (35), we confirmed the expression of VEGF by isolated stromal cells that are devoid of macrophages or other leukocytes (22). As seen in the nonhuman primate, VEGF expression

was greatest during the secretory phase, a time when endometrial endothelial cell proliferation is maximal (36), and endometrium has the greatest angiogenic activity (37).

Regulation of VEGF by ovarian steroids

Consistent with our *in vivo* observations, we found that human endometrial stromal cells in culture synthesize and secrete VEGF. Hybridization observed at approximately 4.4 and 4.2 kb may represent differential mRNA splicing (14). Variations in transcript splicing may be one way in which VEGF activity is regulated. Reverse transcription-PCR, using transcript-specific primers, suggested that multiple transcripts are expressed in uterine tissue (38). The steady state expression of VEGF mRNA was increased by E_2 and MPA *in vitro* in the present studies. As time-course studies showed a near-maximal increase in mRNA as early as 1 h after the addition of E_2, this reproductive steroid appears to regulate VEGF gene transcription directly. The rapidity of this response suggests that VEGF may be a primary response gene for reproductive steroids in the endometrium (39, 40). Despite the fact that consensus sequences for estrogen or progesterone response elements are not found in the 3.4-kb 5′-promoter region of the VEGF gene, several half-palindromes are present (14, 38). These or other variations of hormone response elements may be involved in the observed steroid-induced increase in VEGF mRNA. Increases in steady state mRNA also may be the result of transcript stabilization by reproductive steroids (39). Effects of steroids on cell membranes also might contribute to the rapid effects observed (41, 42).

Similar to our results in primary human endometrial cells, Charnock-Jones *et al.* (15) reported that E_2 treatment of an endometrial carcinoma cell line, HEC 1-A, increased steady state levels of VEGF mRNA in a time-dependent manner. They observed a 6-fold increase in VEGF-specific hybridization, but this did not occur until after 20 h of incubation with steroid. The differences between these reports may be explained by different regulatory processes in transformed endometrial carcinoma cell lines compared to primary endometrial cells. Cullinan-Bove and Koos (38) reported a rapid VEGF mRNA response in whole rat uteri after the administration of E_2 *in vivo*. By quantitative reverse transcription-PCR, they observed an increase in VEGF mRNA within 1 h, with a maximum 8-fold or greater increase after 2 h of estrogen treatment. Similar to our findings in isolated human endometrial cells, progesterone treatment also increased rat uterine VEGF mRNA expression (38). As RNA was extracted from whole rat uteri, their study may reflect the synthesis of VEGF by many cell types, including myometrial smooth muscle cells (43), fibroblasts, and macrophages in addition to endometrial cells. Our finding of increased concentrations of VEGF in endometrial cell-conditioned medium after the addition of E_2 and E_2 and MPA combined confirms that secreted protein parallels the observed increases in VEGF transcripts.

VEGF expression in endometriosis

As VEGF is secreted by human endometrial cells, and its expression is increased by E_2, we proceeded to investigate a possible role for VEGF in the pathogenesis of endometriosis, an angiogenesis-dependent, estrogen-sensitive disease. Using a sensitive bioassay, Oosterlynck *et al.* (24) showed that there was a positive vasculogenic reaction in 58.3% of peritoneal fluid samples from women with endometriosis, but in only 12.5% of controls. The identity of the angiogenesis-inducing factor(s) in peritoneal fluid of women with endometriosis is unknown. We identified VEGF in peritoneal fluid and determined that the concentrations of this secreted angiogenic factor are significantly higher in women with moderate to severe endometriosis than in women with minimal to mild or no disease. VEGF was localized predominantly in glandular epithelium of endometriosis lesions, with a pattern of expression similar to that seen in eutopic endometrium. The likely sources of peritoneal fluid VEGF are pelvic endometriosis implants or peritoneal macrophages. Peritoneal macrophages are increased in women with endometriosis (44) and are known to express VEGF (35). Greater VEGF activity associated with an increase in endometrial angiogenesis could be one determinant of the successful implantation and growth of endometriosis.

The present studies demonstrate that an angiogenic factor, VEGF, expressed in eutopic and ectopic endometrium responds to ovarian steroids both *in vivo* and *in vitro*. A better understanding of factors that regulate the endometrial vasculature is likely to lead to more effective therapy for many pathological states, including endometriosis, dysfunctional uterine bleeding, and endometrial cancer.

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