0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(3):1089–1095

doi: 10.1210/jc.2003-031406

Antiangiogenesis Therapy for Endometriosis

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It is known that angiogenesis is of pivotal importance for the development of endometriosis. However, in the treatment of endometriosis patients, prevention of endometriosis lesion development only will not be sufficient as a therapy. Treatment options, aimed at interfering with established lesions, have to be developed. In this study we evaluated whether inhibition of angiogenesis by angiostatic therapy is also effective in antagonizing the sustentation of endometriosis. We evaluated the effect of the angiostatic compounds antihuman vascular endothelial growth factor, TNP-470, endostatin, and anginex on the growth of established endometriosis lesions in the nude mouse model. We show that human endometrium in the proliferative endometrium is highly angiogenic and that vascular endothelial growth factor-A is the most important angiogenesis promotory factor. The angiostatic compounds significantly decreased microvessel densities and the number of established endometriosis lesions. In the remaining lesions, the number of pericyte-protected vessels is not different in control and treated mice; however, the number of unprotected vessels was significantly reduced in the groups treated with the angiostatic agents. Our data demonstrate that inhibitors of angiogenesis effectively interfere with the maintenance and growth of endometriosis by inhibiting angiogenesis. This suggests that the use of angiostatic agents may be promising as a therapy for endometriosis. (*J Clin Endocrinol Metab* 89: 1089–1095, 2004)

ENDOMETRIOSIS, DEFINED AS functioning endometrium outside the uterine cavity, is found primarily in peritoneum, ovary, and rectovaginal septum. Women suffering from endometriosis may present with chronic pelvic pain, dysmenorrhea, dyspareunia, and subfertility. The prevalence of endometriosis in women with pelvic pain and/or subfertility is estimated to be between 20–90%; thus, it is one of the most encountered benign gynecological problems (1). Endometriosis is believed to be the result of implantation of retrogradely shed endometrium during menstruation (2). Endometrium has the capacity to adhere, attach, and implant ectopically (3, 4). For the survival of endometrium in an ectopic location, the acquisition of an adequate blood supply is essential. Endometrium has angiogenic potential (5), and endometriotic lesions are larger in areas with a rich blood supply (6). This suggests that angiogenesis is a prerequisite for the development of endometriosis.

Angiogenesis is a sequence of events that is fundamental to a broad array of physiological events in the body, including embryogenesis, the menstrual cycle, and wound healing. Angiogenesis is also involved in pathological situations such as tumor growth, atherosclerosis, chronic inflammation, and endometriosis (7). The use of angiostatic agents promises to provide a new therapeutic option for some of these pathological processes. The search for inhibitors of angiogenesis has mainly concentrated on controlling two of the processes involved in angiogenesis: endothelial cell (EC) growth and EC adhesion (8–10). Targeting drugs to ECs may hold promise for the treatment of endometriosis, because ECs are more accessible than other cells to pharmacological agents delivered via the blood. In addition, ECs are genetically stable and thus are not easily mutated into drug-resistant variants (10).

Assuming that angiogenesis is of pivotal importance in the pathogenesis of endometriosis, angiostatic compounds may interfere with the development of endometriosis lesions, as was illustrated recently (11). However, in clinical practice, women will present with established endometriosis. To treat women who suffer from this disease, prevention of only the development of new lesions will not be sufficient as a therapy. Treatment options have to be developed that are aimed at inhibition of the maintenance and growth of established lesions. Therefore, the aim of this study was to evaluate whether the angiostatic compounds antihuman vascular endothelial growth factor-A (anti-hVEGF), TNP-470, endostatin, and anginex, acting in a broad array of angiogenic mechanisms, were effective inhibitors of established endometriosis lesions. To test the effects of these antiangiogenic agents for this purpose, we used the nude mouse model. Human endometrium can be transplanted into nude mice, and endometriosis lesions that are macroscopically and microscopically similar to human endometriosis lesions come to development (11–15). In our study endometriosis lesions were allowed to form over 3 wk. After this period, administration of angiostatic agents was initiated. We studied the number of endometriosis lesions after 2 wk of treatment with angiostatic agents and evaluated microvessel densities in the lesions.

Abbreviations: CD, Cycle day; EC, endothelial cell; H&E, hematoxylin and eosin; hVEGF, human VEGF; αSMA, α-smooth muscle actin; VEGF, vascular endothelial growth factor; VEGF-R, VEGF receptor; vWF, von Willebrand factor.

We demonstrate that angiogenesis is a prerequisite for the maintenance and growth of endometriosis, and that angiostatic compounds effectively inhibit established endometriosis lesions. This indicates that the use of angiostatic compounds may be promising as a therapy for endometriosis.

Materials and Methods

Endometrium tissue collection and preparation

Proliferative endometrium was collected during laparoscopy in six women who had normal ovulatory cycles. Tissue was collected by transvaginal biopsy using a sampling device (Gynotec, Malden, The Netherlands) on cycle day 6 (CD 6), 7, (n = 3), and 9 (n = 2) of the menstrual cycle. The women were 25–42 yr of age, and indications for laparoscopy were abdominal pains, tubal testing, and sterilization. No gynecological pathology was found in any of the endometrium biopsies.

The use of human endometrium was approved by the institutional ethical review committee of University Hospital Maastricht. All women gave written informed consent. After collection, blood was removed, and endometrium was kept in serum-free DMEM/Ham's F-12 culture medium. From each endometrial biopsy, several pieces of tissue were embedded in paraffin wax. After hematoxylin and eosin (H&E) staining, tissue integrity was evaluated, and the day of the menstrual cycle (cycle day) was histologically confirmed by a pathologist.

After collection, the tissue in serum-free DMEM/Ham's F-12 culture medium was placed on ice and minced in small pieces approximately 2–3 mm^3 in size. On two separate occasions, two endometrial biopsies were collected on the same day (CD 6 and 7; CD 7 and 9). After preparation, the tissue pieces from CD 6 and 7 were injected in a total of 22 mice, and the tissue pieces from CD 7 and CD 9 were injected in a total of 35 mice. Of the 35 mice, eight were used to evaluate lesion vascularization development for the duration of the experiment. The other two biopsies (CD 7 and 9) were used for RNA isolation and angiogenic profiling.

Real-time RT-PCR

To investigate the angiogenic profile of proliferative human endometrium, real-time RT-PCR was performed. Total RNA was isolated from human proliferative endometrium tissue using the RNeasy RNA isolation kit (Qiagen, Chatsworth, CA) according to the supplier's protocol. Column deoxyribonuclease treatment with the ribonuclease-free deoxyribonuclease set (Qiagen) was used to remove any genomic DNA. The purity and integrity of the RNA were checked by gel electrophoresis according to standard procedures. Glyceraldehyde-3-phosphate dehydrogenase PCR was performed to assure that all genomic DNA has been removed.

One microgram of total RNA was reverse transcribed for 1.5 h at 42 C with 600 U Moloney murine leukemia virus reverse transcriptase (Promega Corp., Madison, WI) in 20 μl 1× first strand buffer (Promega Corp.), and 1 mM deoxy-NTPs in the presence of 40 U of the ribonuclease inhibitor RNasin (Promega Corp.) and 0.5 μg random primers (Promega Corp.). Real-time RT-PCR was carried out on approximately 30 ng cDNA (assuming a 1:1 conversion) in an ABI PRISM 7700 Sequence Detection System apparatus (PE Applied Biosystems, Foster City, CA) in a 25-μl volume containing 1× SYBR Green PCR master mix (PE Applied Biosystems) and 500 nM of the forward and reverse primers using the following PCR profile: 10 min at 95 C, followed by 50 cycles of 15 sec at 95 C and 1 min at 60 C. Primers used for real-time RT-PCR were targeted against β-actin; cyclophilin A; VEGF-A, -B, -C, and -D; angiopoietin-1, -2, and -3; basic fibroblast growth factor; placental growth factor; VEGF receptors 1, 2, and 3 (VEGF-R1, VEGF-R2, VEGF-R3); neuropillin 1 and 2; and tyrosine kinase receptors 1 and 2. The sensitivity of all primer sets was confirmed on a dilution series of clone cDNA fragments. Each primer pair had a linear amplification rate within the expected slope of approximately −3.3, down to 1 fg template DNA (Thijssen *et al.*, manuscript in preparation). The parameter cycle threshold was defined as the cycle number at which the fluorescent signal passed a fixed value, and the expression of each target gene was normalized to the expression of the control genes.

Reagents

HuMV833, a humanized anti-hVEGF-A antibody (provided by Protein Design Laboratories, Fremont, CA) (16), TNP-470 (AGM-1470, provided by Takeda Chemical Industries, Osaka, Japan) (17), endostatin (provided by Entremed, Inc., Rockville, MD) (18), and anginex (19) were used at the effective dosages reported. As TNP-470 is a strong general angiogenesis inhibitor, we used this agent as a positive control for angiostasis.

Nude mouse model

Fifty-seven female mice (Swiss *nu/nu*, Charles River, Maastricht, The Netherlands) were individually housed in autoclaved cages and bedding, in laminar flow filtered hoods. The animal room was maintained at 26 C with a 12-h light, 12-h dark cycle, and mice were fed *ad libitum* with autoclaved laboratory rodent chow and acidified water. All handling was performed in laminar flow filtered hoods. A mixture of ketamine/xylazine (100 mg/kg ketamine and 10 mg/kg xylazine; Eurovet, Bladel, The Netherlands), sc injected in a volume of 0.1 ml/10 g bodyweight, was used to anesthetize mice before invasive procedures, using sterile instruments. The Maastricht University ethical review committee for animal experiments approved the use of mice for this study.

At the age of 5 wk, sterile 60-d release capsules containing 18 mg 17β-estradiol (Innovative Research of America, Sarasota, FL) were placed sc in the neck of each animal. According to the manufacturer's information, capsules provide continuous release of hormone at serum concentrations of 150–250 pmol/liter, in the range of physiological levels in mice during the estrous cycle (20). This stable physiological level of estrogen promotes the growth of transplanted human endometrium and eliminates intermouse differences related to various stages of the estrous cycle.

Four days after insertion of the estrogen pellet, an entrance was made to the peritoneal cavity in the midline in the lower abdomen with an 18-gauge needle, and with the help of a pipette, 10 fragments of fresh human endometrium in 200 μl sterile PBS were inoculated ip to mimic the situation after retrograde menstruation in women. Another entrance was made sc through the skin in the flank, and 10 fragments of fresh human endometrium were pipetted sc to enlarge the probability of recovery. Endometriosis lesions were found equally at both locations, suggesting that both sc and ip inoculations of human endometrium fragments result in the formation of endometriosis lesions in nude mice.

The endometrium collected on CD 6 and 7 was transplanted in 22 mice. The mice received the following treatments: control (n = 5), anti-VEGF (n = 5), endostatin (n = 4), TNP-470 (n = 4), or anginex (n = 4). The endometrium collected on CD 7 and 9 was transplanted in 35 mice, which received the following treatments: control (n = 18), anti-VEGF (n = 6), endostatin (n = 5), or anginex (n = 6). TNP-470 was not included in the second experiment. Eight of the control mice from the second experiment were used to study normal lesion development and vascularization in time. Two control mice were killed by cervical dislocation 1, 2, 3, and 4 wk after implantation of the endometrium fragments.

The preparation and transplantation of the tissue took about 3 h. The mice were prepared in groups of four or five at a time. After all mice received endometrial tissue, the mice were distributed among the treatment groups. To alleviate variation as a result of the tissue preparation time, we composed the treatment groups of mice from all preparation groups wherever this was possible.

Because of the known negative side-effects on mice, this group was kept as small as possible. Apart from studying endometriosis-like lesion formation, we did not study morphometric aspects or vascularization in this small subgroup of mice. Three weeks after implantation of endometrium fragments, administration of angiostatic agents was initiated. Anti-hVEGF (3 mg/kg·d) (16) and TNP-470 (20 mg/kg every 2 d) (17) were administered sc. Endostatin (2 mg/kg·d) (18) and anginex (8 mg/kg·d) (19) were administered by miniosmotic pumps (Alzet, DURECT Corp., Cupertino, CA) placed sc on the back of the animals. To control mice, 100 μl normal saline were administered daily, sc. Five weeks after implantation of the endometrium fragments, all mice were killed.

Analysis of endometriosis lesions and vascularization in nude mice

To evaluate endometriosis lesions and vascularization, the abdominal skin was opened, and the abdominal sc region, the peritoneum, and visceral organs were examined under a binocular microscope. Uterus and lesions with possible endometriosis were removed, fixed in 10% buffered formalin, and embedded in paraffin wax. Paraffin sections (4 μm) were cut from the entire specimen (150–200 sections), and sections were stained with H&E or used for immunohistochemistry. Histology of endometriosis lesions was evaluated by a pathologist specialized in gynecology and a laboratory animal pathologist.

The number of von Willebrand factor (vWF)-stained vessels (as described below) in the lesion was counted under ×200 magnification, and the number of vessels per square millimeter lesion was calculated. Under identical circumstances, the number of mature vessels surrounded by α-smooth muscle actin (αSMA)-positive cells (as described below) was calculated. To calculate the number of newly developed vessels, the number of αSMA-stained vessels was subtracted from the number of vWF-stained vessels. Each lesion was examined twice, and the average of the counts was taken.

Immunostaining

Immunostaining for VEGF on human endometrium was performed using an antibody against VEGF (polyclonal, 1:200; Novocastra Laboratories Ltd., Newcastle upon Tyne, UK). The human origin of endometriosis lesions was confirmed by immunostaining using an antibody against pancytokeratin (MNF 116, monoclonal, 1:500; DAKO, Glostrup, Denmark), specifically staining human epithelial cells. Blood vessels were stained by immunohistochemistry using an antibody against vWF (polyclonal, 1:1000; DAKO), and smooth muscle cells surrounding mature vessels were stained using an antibody against αSMA-fluorescein isothiocyanate (monoclonal, 1:3000; Sigma-Aldrich Corp., St. Louis, MO). Antibody binding was visualized with Envision (anti-VEGF, MNF 116, vWF) or with antifluorescein isothiocyanate-horseradish peroxidase (αSMA)-labeled secondary antibody and 3,3′-diaminobenzidine as a chromogen. Sections were counterstained with hematoxylin and mounted with coverslips.

Statistical analysis

Descriptive statistics (median, range, and percentiles) were calculated for each experimental group. Differences in number of endometriosis lesions as well as differences in microvessel densities between groups of mice were compared using the nonparametric Mann-Whitney U test. $P < 0.05$ was considered statistically significant. Microvessel densities were counted blindly, twice by one observer, and once by a second observer under identical circumstances. The average of the counts was taken. Intra- and interobserver variabilities were between 5–10%.

Results

Human endometrium tissue is highly angiogenic

The angiogenic profile of proliferative endometrium tissue was investigated by real-time RT-PCR (quantitative RT-PCR, Fig. 1A). VEGF-A was found to be the predominant angiogenic factor. This was confirmed by detection of VEGF at the protein level with immunohistochemistry (Fig. 1, B and C). Quantitative RT-PCR revealed that basic fibroblast growth factor and angiopoietin-2 expressions were also relatively high, whereas VEGF-B and -C, placental growth factor, and angiopoietin-1 were expressed at low levels. VEGF-D and angiopoietin-3 were virtually absent. Interestingly, whereas VEGF-R1 and -2 were expressed moderately, neuropillin-1 was the predominant receptor mRNA related to angiogenesis in the endometrial tissue (Fig. 1A).

Development of endometriosis in mice is inhibited by angiostatic agents

Human endometrium fragments injected sc and in the peritoneal cavity of estrogen-supplemented athymic mice gave rise to endometriosis lesions at both locations in more than 95% of mice. In a longitudinal study, two or three lesions were found in each mouse when mice were killed 1, 2, 3, 4, or 5 wk after inoculation, with an average of 2.5 lesions/mouse (not significant). This indicates that engraftment of endometrium results in quick and stable development of endometriosis lesions. In time, an increase in microvessel density was observed when determined by vWF staining,

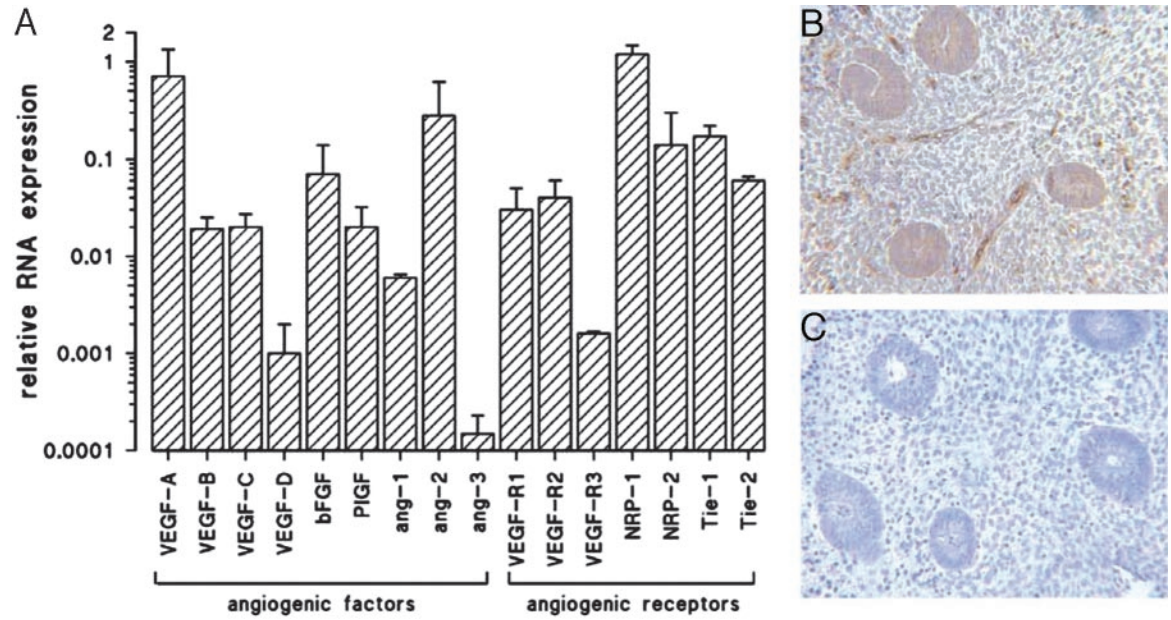

FIG. 1. Angiogenesis profile of human endometrium. A, Quantitative real-time RT-PCR for endothelial growth factors. B, Immunostaining for hVEGF in proliferative human endometrium shows that VEGF is expressed abundantly. C, Negative control staining in the same proliferative human endometrium specimen. Magnification, ×200.

increasing from 5 vessels/mm^2 lesion after 1 wk (range, 3–8) to 13.5 vessels/mm^2 lesion after 5 wk (range, 6–41; $P <$ 0.005).

The angiostatic agents did not appear to affect the overall health of the mice, as the body weights of the mice in the different treatment groups were not significantly different. The behavior of the mice during treatments was normal as well.

After 5 wk, in control mice an average of 2.5 lesions were present. Treatment with anti-hVEGF resulted in a significant decrease in the number of lesions observed per mouse ($P <$ 0.05; Fig. 2D). This indicates that VEGF is a key player in the endometrium-derived signals. In addition to anti-hVEGF, mice were treated with three other angiogenesis inhibitors, *i.e.* the fungus-derived antibiotic TNP-470 (21) and the specific angiogenesis inhibitors endostatin (22) and anginex (19, 23, 24). All three compounds significantly inhibited the number of endometriosis lesions present in mice ($P < 0.05$; Fig. 2D).

Morphology was assessed by a laboratory animal pathologist and by a pathologist specialized in gynecology. A pancytokeratin staining specifically for human epithelium confirmed the human origin of the endometriosis lesions (Fig. 2C). All endometriosis lesions showed heterogeneous morphology. Part of the lesions presented as tubal metaplasia (endosalpingeosis), consisting of metaplastic epithelium surrounded by fibrotic tissue instead of stroma. This morphology equals morphology often seen in rectovaginal endometriosis in women. Other parts of the lesions contained more

TABLE 1. Numbers of vWF-stained vessels and mature αSMA-stained vessels, and the difference between vWF- and αSMA-stained vessels in endometriosis lesions in nude mice per square millimeter of lesion

	vWF-stained vessels	αSMA-stained vessels	vWF-αSMA-stained vessels
Control	13.5 (1–52)	8 (1–29)	4 (0–30)
Anti-hVEGF	8.5 (1–18)[a]	4 (1–17)	1 (0–8)[a]
Endostatin	5 (3–8)[a]	5 (2–8)	0 (0)[a]
Anginex	5 (3–10)[a]	5.5 (3–9)	0 (0–9)[a]

Values are medians (range).
[a] $P < 0.05$ compared to control.

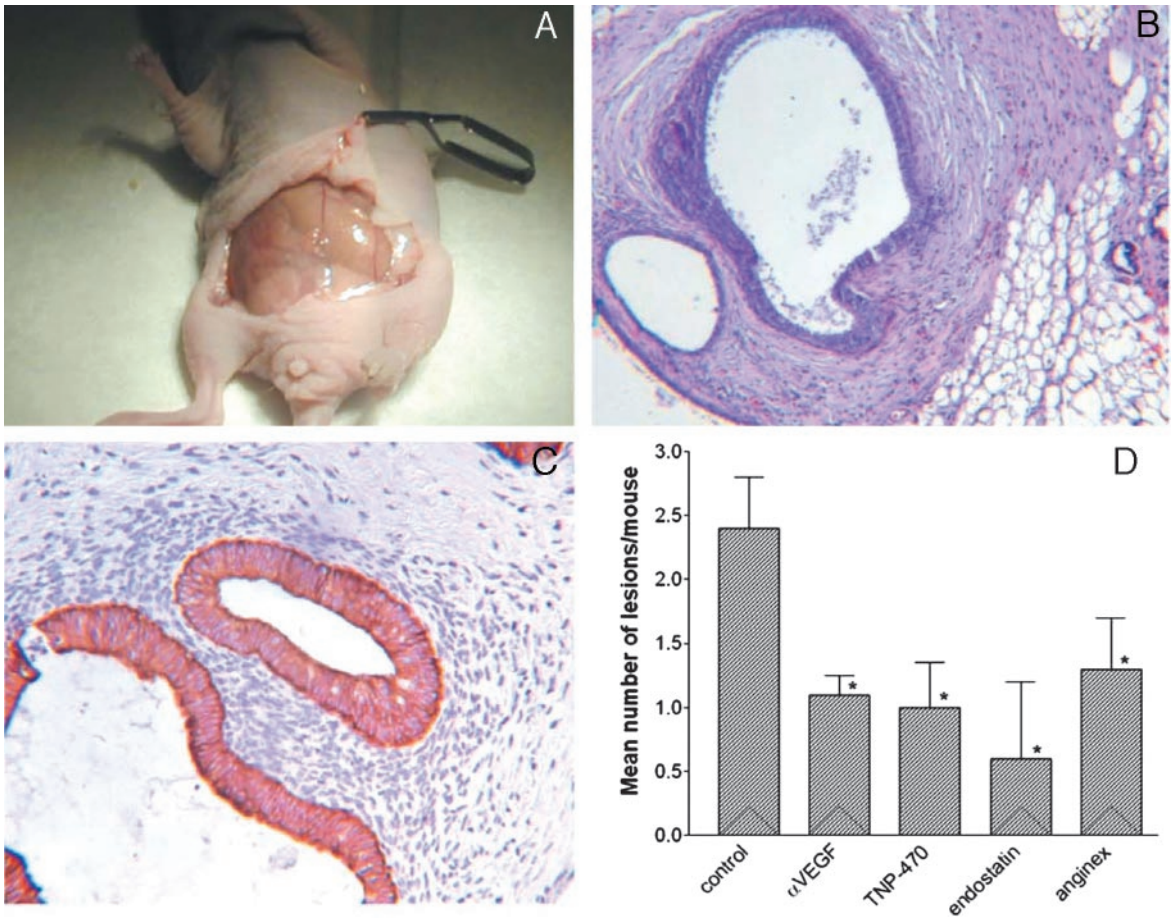

FIG. 2. Formation and morphology of endometriosis lesions in nude mice after administration of angiostatic agents. A, Intraperitoneal lesion in nude mouse (control). The endometriosis lesion is visible through the peritoneum, forming white nodules in the mouse abdomen. B, Representative cross-section of an sc endometriosis lesion in a nude mouse (H&E staining). Note the heterogeneous morphology of the endometrium with tubal metaplasia surrounded by fibrosis, and highly cylindrical epithelium covered with typical endometrial stroma (control mouse; magnification, ×200). C, Endometrium glands stained with a specific human pancytokeratin antibody (MNF 116) in a peritoneal endometriosis lesion, showing that the endometriosis lesion is of human origin (control mouse; magnification, ×300). D, Number of endometriosis lesions in nude mice after treatment with angiostatic agents.

typical, highly cylindrical endometrial epithelium surrounded by stroma (Fig. 2B), as is common for peritoneal endometriosis in women. Histology did not differ between the groups, and no relation was found between histology and the vascular density of the lesion.

Antiangiogenic agents suppress neovascularization in lesions in nude mice

Microvessel density, determined on the basis of vWF-positive vessels, was suppressed in endometriosis lesions in mice treated with angiostatic agents ($P < 0.05$; Table 1) compared with control mice. Endostatin was found to be the most effective inhibitor of microvessel density. Both competition of VEGF and treatment with anginex led to a reduction of microvessel density. Similar results were observed for endometriosis lesions in mice treated with TNP-470, but these results were left out of Table 1 due to the small number of lesions. When vessel density was determined using αSMA antibodies, quantifying mature vessels, no differences were observed between the treatment groups. Enumeration of the number of vWF^+ αSMA^- vessels, which can be considered the newly formed vessels, revealed a suppressed appearance in all groups treated with angiogenesis inhibitors ($P < 0.05$; Table 1). Figure 3

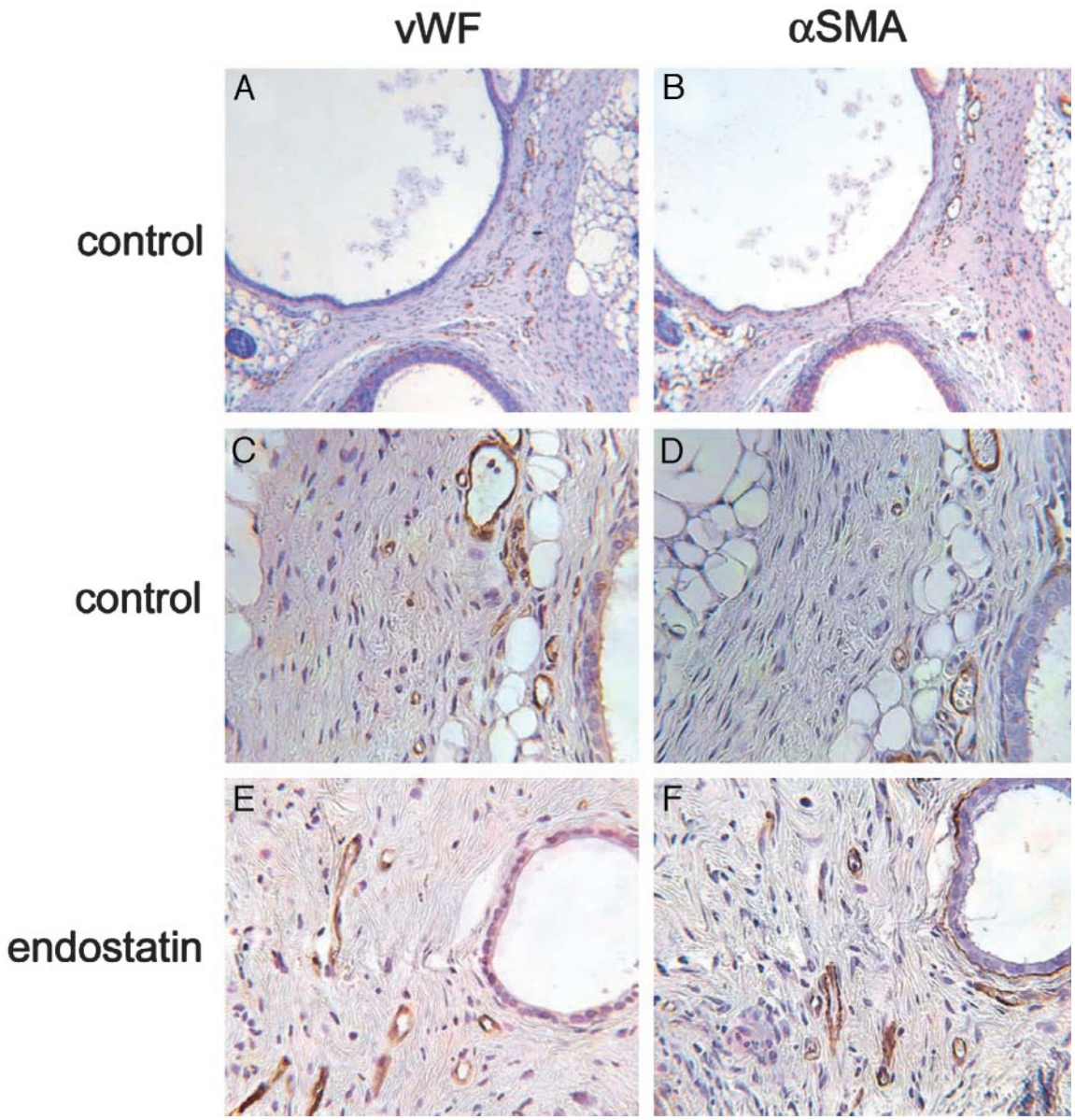

FIG. 3. vWF and αSMA staining of vessels in endometriosis lesions in nude mice. In control mice, not all vWF-positive vessels (A and C) are αSMA positive (B and D), indicating that mature as well as newly formed vessels are present in endometriosis lesions in control mice. A and B, Overview of a representative endometriosis lesion (magnification, ×200). C and D, Detailed representative pictures are shown, in which vWF-positive and αSMA-negative vessels are present (magnification, ×300). In mice treated with endostatin, all vWF-positive vessels (E) are αSMA positive (F), indicating that only mature vessels remain and that no new vessels develop as a consequence of treatment with antiangiogenic agents (magnification, ×300).

shows the difference in vWF- and αSMA-stained vessels in an endometriosis lesion in mice.

Discussion

The aims of the present study were to assess whether the maintenance and growth of endometriosis lesions are dependent on angiogenesis and whether angiogenesis inhibition is a therapeutic possibility for endometriosis. The current data demonstrate that the maintenance and growth of endometriosis are strongly dependent on angiogenic processes, and that angiostatic agents are able to inhibit established endometriosis lesions. These data were obtained from an *in vivo* human xenograft animal model using angiogenesis inhibitors for intervention.

Real-time RT-PCR analysis of proliferative endometrium demonstrated that VEGF-A is the most abundantly expressed angiogenic factor in human endometrium, which suggests a pivotal role for VEGF in endometrium biology and confirms earlier results reported by others (11, 25–28). Although the expression of VEGF was present in both epithelial and stromal cells of the endometrium, dominant expression was observed in microvessels within the endometrium tissue, which is likely to be receptor-bound VEGF. The presence of both VEGF-R1 and -R2 as well as the extremely high expression of neuropillin-1, which is a coreceptor of VEGF-A, supports the fact that VEGF-A is the most important angiogenic factor in endometrium tissue.

In a recently published study, the effect of angiogenesis inhibition was studied in nude mice. VEGF-A inhibitors were administered immediately after implantation of cultured human endometrium fragments (11). The researchers observed impaired lesion formation, and they concluded that angiostatic agents may be effective in the treatment of endometriosis. However, angiostatic agents were applied immediately after implantation of human endometrium, when endometriosis lesions had not yet developed. Therefore, development of endometriosis lesions was prevented in this study, but no therapy for established endometriosis lesions was applied. In our study we used uncultured human endometrium to avoid adverse effects. Moreover, we initiated angiostatic treatment 3 wk after implantation of human endometrium fragments. In these 3 wk, endometriosis lesions were allowed to establish. The start of angiostatic therapy after endometriosis lesions have been established seems to be a more realistic study design. In the clinical situation, treatment is initiated in women after endometriosis has been diagnosed, at which point endometriosis lesions have already been present for a period of time. In addition, we applied not only an anti-VEGF strategy, but also a number of angiostatic agents affecting a broader array of angiogenesis mechanisms. Not only anti-VEGF, but also TNP-470, endostatin, and the newly developed antiangiogenic agent anginex turned out to effectively interfere with established endometriosis lesions. We found that the number of endometriosis lesions after 5 wk of incubation was significantly lower in mice treated with angiostatic agents compared with the control group. The observed effects are ascribed to the antiangiogenic capacity of these agents. Interestingly, physiological angiogenesis in the mice appeared not to be affected by the angiogenesis inhibitors. Surgery in mice was performed without later bleeding complications, and no differences were observed in visual aspects of wound healing between control mice and mice treated with angiostatic agents. Moreover, vascularization of the uteri of mice in different groups did not differ (data not shown). These observations suggest that normal angiogenesis was not affected by the application of angiostatic agents.

The morphology of endometriosis lesions in all treatment groups was diverse, with typical endometrium glands and stroma as well as tubal metaplasia with large cysts and flattened epithelium. The observed morphology was similar to the morphology that is often seen in endometriosis in women. We found no relation between the morphology and the vessel density of the lesions.

Vessel density, based on staining with vWF antibody, was significantly lower in mice treated with angiostatic agents compared with control mice. The angiostatic agents may alter the permeability of the vessels and cause edema, which may have a significant effect on the apparent vascular density when expressed per square millimeter. However, the number of αSMA-positive, mature vessels did not differ between the groups, which indicates that the vessels that have regressed were the newly developed ones and not the smooth muscle cell-protected, mature vessels. Apparently, development of new blood vessels remains of pivotal importance for the maintenance and growth of endometriosis. This is also obvious from clinical observations, where newly developed, red peritoneal endometriosis lesions are vascularized by many small blood vessels with mitotically active endothelial cells (6) and relatively small numbers of smooth muscle cell-protected adult blood vessels (29). With age, the lesions evolve into black, hemorrhagic lesions, with larger blood vessels (6) that have a higher vessel maturation index, suggesting that the number of smooth muscle cell-protected blood vessels has increased. However, unprotected vessels remain present (29). Therefore, angiostatic therapy may delay the progression of established endometriosis.

To date, endometriosis is hormonally treated, aimed at achieving a hypoestrogenic state. Hormonal therapy only suppresses symptoms, but will not eradicate the ectopic implant. Moreover, there are significant side-effects. Long-term hormonal therapy, therefore, is not an attractive option. Alternatively, endometriosis can be treated surgically. Conservative surgery consists of ablation of endometriosis lesions, resulting in pain relief, but symptoms recur in time in a majority of women. Radical surgery includes removal of uterus and/or ovaries, giving more permanent symptom relief, but resulting in the end of reproductive life. An effective therapeutic agent for endometriosis would be a compound that not only prevents the development of endometriosis lesions, but would also be effective against the growth of established lesions. In cancer, ECs have been shown to play a pivotal role in tumor cell survival and growth. In analogy with tumor growth, endometriosis is shown to be highly dependent on angiogenesis, which makes the achievements in the field of cancer research applicable to endometriosis. Recently, striking results have been achieved with Avastin (Genentech, San Francisco, CA), a humanized anti-VEGF antibody. This approach of neutralizing VEGF pro-

vided the first proof of concept that antiangiogenesis is applicable in humans (30, 31). The major role of VEGF in endometriosis may predict the success of Avastin in endometriosis.

In conclusion, we have shown that angiogenesis is a prerequisite for the maintenance and growth of endometriosis. Our data demonstrate for the first time that different kinds of inhibitors of angiogenesis effectively interfere with established endometriosis lesions. Therefore, we favor antiangiogenesis therapy for clinical testing for endometriosis. When symptoms of endometriosis have been treated by hormones or surgery, antiangiogenic agents may be applied to eradicate residual and/or microscopic endometriosis.

Acknowledgments

We acknowledge M. Gijbels, Ph.D., animal pathologist, and N. Sieben M.D., gynecological pathologist, for evaluation of the morphology of the mouse sections; J. Cleutjens, Ph.D., for designing the Quantimet computer program for the morphological characterization of endometriosis lesions; and J. P. Schouten and Q. Theunissen for excellent technical assistance.

Received August 11, 2003. Accepted December 8, 2003.

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This work was supported by an unrestricted grant from Ferring BV (Hoofddorp, The Netherlands; to A.W.N.) and by the Stichting Technische Wetenschappen (Grant MPG-5456; to A.W.G.).

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