Suppression of Matrix Metalloproteinases Inhibits Establishment of Ectopic Lesions by Human Endometrium in Nude Mice

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Abstract

Matrix metalloproteinases of the stromelysin family are expressed in the human endometrium as a consequence of cellular events during the menstrual cycle that require extracellular matrix remodeling. We have recently documented the presence of these enzymes in lesions of endometriosis, a benign disease that presents as persistent ectopic sites of endometrial tissue, usually within the peritoneal cavity. Endometriosis can develop after retrograde menstruation of endometrial tissue fragments, and establishment of ectopic sites within the peritoneal cavity requires breakdown of extracellular matrix. To examine whether matrix metalloproteinases might contribute to the steroid-dependent epidemiology and cellular pathophysiology of endometriosis, we have developed an experimental model of endometriosis using athymic nude mice as recipients of human endometrial tissue. Our results demonstrate that estrogen treatment of human endometrial tissue in organ culture maintains secretion of matrix metalloproteinases, and promotes establishment of ectopic peritoneal lesions when injected into recipient animals. In contrast, suppressing metalloproteinase secretion in vitro with progesterone treatment, or blocking enzyme activity with a natural inhibitor of metalloproteinases, inhibits the formation of ectopic lesions in this experimental model. (*J. Clin. Invest.* 1997. 99:2851–2857.) Key words: matrilysin • stromelysin • endometriosis • progesterone • TIMP-1

Introduction

The matrix metalloproteinases (MMPs)[1] constitute a related family of proteolytic enzymes that not only play important roles in the remodeling of extracellular matrix in normal tissues, but also contribute to pathologies such as tumor invasion (1). The human endometrium is a unique tissue that undergoes regular cycles of growth, differentiation, tissue breakdown, and repair throughout a woman's reproductive life. Not unexpectedly, the expression of numerous MMPs has been detected during the dynamic tissue responses that make up the normal menstrual cycle (2–6). The expression of endometrial MMPs occurs in association with growth-related tissue remodeling as well as with menstrual breakdown and repair, but specific messages for these enzymes in either stromal or epithelial cells are not detectable during the early-mid secretory phase of the cycle in vivo (5, 6). Given the role of MMPs during both normal and pathologic processes in many tissues, our research group has focused on steroid-dependent regulation of members of the stromelysin family in the normal endometrium (5–8) compared to the disease endometriosis (9).

In normal endometrium, a number of laboratories have confirmed the ability of progesterone to suppress stromelysin-1 (MMP-3) and stromelysin-3 (MMP-11) mRNA expression and MMP-3 protein secretion in vitro (7, 8, 10). The expression of MMP-3 and MMP-11 can be suppressed by progesterone treatment in isolated stromal cells in vitro (7), but epithelial-specific matrilysin (MMP-7) expression does not appear to be suppressed directly by progesterone alone. Progesterone has been shown to increase stromal cell expression of transforming growth factor-β in vivo, and this growth factor appears to be required for progesterone suppression of MMP-7 in vitro (8). While studies to date indicate that the rather complex mechanisms by which steroids can mediate MMP expression are a critical component of normal endometrial function, little is known in regard to the role that regulation of these enzymes might play in the disease processes of endometriosis. The expression of MMP-3, MMP-7, and tissue inhibitor of metalloproteinase-1 (TIMP-1), however, have each been documented in lesions of endometriosis (for review see reference 9).

Endometriosis is a benign disease that presents as pleomorphic lesions of endometrial tissue containing both glands and stroma growing at sites outside the uterine corpus. The exact etiology of this disease is controversial, but endometriosis occurs infrequently outside of the reproductive years, and appears to develop principally from the ectopic implantation of endometrial tissue entering the peritoneal space at the time of menstruation (11, 12). Additionally, numerous experimental observations demonstrate that introduction of endometrial fragments into the peritoneal space of women (13, 14) and nonhuman primates (15) can result in the development of endometriosis. While the precise mechanisms by which displaced endometrial tissue leads to ectopic lesions remain speculative, MMPs are highly expressed in the endometrium at the time of menstruation as well as during the subsequent period of endometrial repair and regrowth (5, 6). The initial establishment

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1. *Abbreviations used in this paper:* MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

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of this disease is clearly an invasive event requiring extracellular matrix breakdown (16), and extensive literature supports an active role for MMPs in the invasive behavior of normal and neoplastic cells (17). Understanding the role of MMP regulation by steroids in the pathophysiology of endometriosis is an important area of concern since ectopic sites of this disease can be as invasive, widely disseminating, and difficult to eradicate as a malignancy.

Exposure to estrogen appears to predispose certain women to the development of endometriosis, while progesterone provides a degree of protection against the development or recurrence of the disease (18). In this study, we have used ovariectomized, athymic (nude) mice as recipient animals for the establishment of ectopic human endometrial lesions to examine the potential role of MMP expression and activity in the establishment of this disease. In developing this experimental model, we also sought to determine whether the in vitro regulation of endometrial MMP secretion by steroids might affect the subsequent ability of human tissue to establish ectopic lesions in mice. To further implicate the action of secreted MMPs in this disease process, we examined the ability of TIMP-1 treatments to modulate the establishment of ectopic lesions in our experimental model. Results of these experiments suggest that steroidal regulation of the secretion of MMPs by human endometrium is critical to the ability of endometrial tissue fragments to establish ectopic sites of growth.

Methods

Acquisition of human tissue

Samples of endometrial tissue were obtained during the proliferative interval (days 9–12) of the menstrual cycle from a donor population of women (ages 21–45) exhibiting regular menstrual cycles. An endometrial thickness of > 9 mm was confirmed by vaginal ultrasound before each biopsy, and a serum progesterone level of < 1.0 ng/ml was necessary for inclusion in this study. Biopsies were obtained from the fundus region of the endometrial cavity with an endometrial suction curette (Pipelle®; Unimar, Inc., Wilton, CT), and were washed immediately in prewarmed (37°C) phenol red–free Dulbecco's modified Eagle's medium/Ham's F-12 medium (DME/F-12) (Sigma Chemical Co., St. Louis, MO) to remove residual blood and mucus. The use of human tissues for this study was approved by Vanderbilt University's Institutional Review Board and Committee for the Protection of Human Subjects.

Explant cultures of endometrium

An important goal of the experiments described in this study was to maintain or redirect the expression and secretion of MMPs in short-term cultures of human endometrium before use in the establishment of an in vivo model of experimental endometriosis. Therefore, endometrial tissues were acquired and carefully sectioned into uniform, 1×2 mm^2 fragments with the aid of a dissecting microscope. Tissue fragments were divided into multiple groups as required for experimental treatments, and were suspended as explant cultures within tissue culture inserts (Millipore Corp., Bedford, MA) near the air/media interface as previously described (7). Each explant culture was maintained under serum-free conditions in DME/F-12 medium supplemented with 1% ITS$^+$ (Collaborative Biomedical Products, Bedford, MA) and 0.1% Excyte (Miles Inc., Kankakee, IL), and was incubated at 37°C in a humidified chamber with 95% air/5% CO_2. Standard steroid treatments of explant cultures included estradiol alone (10 nM) or estradiol plus progesterone (1 and 500 nM, respectively). Control cultures received no steroid supplement, but were otherwise treated in identical fashion. Explant cultures of human endometrial fragments were maintained for 24 h before analysis of MMP secretion or use in the nude mouse experimental model as outlined below. Representative tissues from treatment or control groups were formalin-fixed after 24 h for morphological assessment after standard hematoxylin/eosin staining.

Analysis of MMP secretion in endometrial explant cultures

Secretion of pro-MMP-7 or pro-MMP-3 by endometrial explants was analyzed after [^{35}S]methionine labeling (100 μCi) for 18 h at 37°C in methionine-free medium as previously described (7). Secreted proteins were quantitated by TCA precipitation, and equivalent TCA-precipitable counts ($0.5–1 \times 10^6$ cpm) were selectively immunoprecipitated using antibodies directed against a synthetic peptide corresponding to a deduced sequence in the carboxy terminus of rat prostromelysin-1 (MMP-3; 19), or an internal sequence in human promatrilysin (MMP-7; 20). The resulting complexes were removed with protein A–Sepharose, and were identified by SDS-PAGE and autoradiography.

Experimental model of endometriosis

Nude mice. 5-wk-old athymic (nude), ovariectomized mice were purchased from Harlan-Sprague Dawley Inc. (Indianapolis, IN) and housed on site in previously sterilized cages and bedding. The animal room was maintained at 26.7°C with a 12-h light/12-h dark cycle, and mice were provided with sterile Purina Rodent Chow® and water ad libitum. Metofane® (Pittman-Moore, Toledo, OH) was used to anesthetize animals before any invasive procedures, which were always performed in a laminar flow hood using sterile instruments. Mice were randomly divided into groups, and sterile 60-d release capsules containing either 1.5 mg 17β estradiol or 15 mg progesterone (Innovative Research of America, Mendelein, IL) were inserted subcutaneously at a site just below the scapula. In addition to the experimental groups, a control group of animals was anesthetized, but not implanted with steroid-releasing capsules.

In vitro treatment and in vivo injection of human endometrial tissue. Human endometrial tissue, maintained for 24 h in culture as described above, was washed briefly in sterile PBS before injection into recipient nude mice. Each animal received a single intraperitoneal injection of 8–10 endometrial tissue fragments in 200 μl sterile PBS in a manner similar to that described by Zamah et al. (21). Endometrial tissues were injected using tuberculin syringes and 18-gauge needles at a site on the ventral midline just below the umbilicus. All animals had received a steroid-releasing capsule (or sham operation) 24–96 h before human tissue injections.

Animal death and assessment of ectopic lesions. Nude mice are essentially hairless, and ectopic endometrial lesions developing on the visceral peritoneum can be visualized as early as 2–4 d after injection of human tissue. 10–12 d after receiving human tissue injections, mice were killed and necropsied for signs of endometriotic-like lesions. The peritoneum and visceral organs of animals were examined using a dissecting microscope, and lesions exhibiting the distinctive morphology of endometriosis were removed and immediately placed in 10% ice-cold buffered formalin. Morphological and immunohistochemical characterization of ectopic lesions by standard pathological methods was done to confirm the distinctive histologic characteristics of endometriosis. Any tissues removed that did not contain both glandular and stromal elements (i.e., fibrous adhesions) were excluded from the study. Additionally, the human origin of the lesions removed from mice was confirmed by localization of epithelial membrane antigen using a human-specific antibody (EMA; SeraLabs, Sussex, United Kingdom; distributed by Accurate Antibodies, Westbury, NY) and standard immunohistochemistry techniques (9).

TIMP-1 treatments

A separate set of experiments was conducted as described above in the experimental model of endometriosis, but was designed to block selectively the activity of MMPs with recombinant TIMP-1 (a generous gift from Dr. George Stricklin) (22, 23). For this study, duplicate estradiol-treated explant cultures were established so that one group of tissue also received in vitro treatment with 0.2 μg/ml of TIMP-1 (prepared for use in animals by Synergen, Inc., Boulder, CO). Recipi-

ent mice were similarly divided so that half of the animals received TIMP-1 treatment via injections of 0.2 μg recombinant TIMP-1 in 200 μl sterile PBS at the time of tissue injection and at 4, 8, and 12 h thereafter. Other than the addition of TIMP-1 treatments in vitro only, in vivo only, or both in vitro and in vivo, these experiments were conducted exactly as described above.

Statistical analysis

Linear regression analysis was performed on in vivo experiments (regression analysis computer program; SAS Institute, Cary, NC). Statistical significance was considered to be $P \leq 0.05$.

Results

Steroidal modulation of MMPs in endometrial organ culture

We have previously reported the effects of steroid treatment on MMP-3 and MMP-7 mRNA expression and protein secretion in explant cultures of human endometrium maintained for 72 h in vitro (7, 9). Given the goal of our current study to establish ectopic human endometrial lesions in nude mice after in vitro culture, however, we examined whether or not a shorter in vitro steroid exposure could be used to regulate endometrial MMP secretion. As shown in Fig. 1, *A* and *B*, tissue explants treated with estradiol for 24 h continue to secrete the proenzyme form of MMP-7 and MMP-3, while treatment with estradiol and progesterone substantially suppresses the secretion of both stromal and epithelial-specific MMPs. After either steroid treatment for 24 h, the induced pattern of MMP secretion was maintained for 72 h in the absence of continued steroid exposure in vitro (not shown). After short-term cultures, endometrial tissues were also examined morphologically for phenotypic changes associated with the distinctive response of the endometrium to estrogen or progesterone exposure in vivo (Fig. 2, *A–D*). Endometrial tissues maintained in culture for 24 h with estradiol treatment maintain the normal morphological characteristics of the proliferative menstrual phase, exhibiting predominantly round, tightly compacted glands (Fig. 2, *A* compared to *C*). Endometrial tissue receiving no steroid treatment over 24 h in vitro (controls) remained morphologically similar to the estradiol treatment group (not shown). In contrast to the control or estradiol treatment, endometrial cultures treated with a combination of estradiol and progesterone for 24 h acquired the distinctive morphology associated with the secretory phase in vivo. Specifically, tissue treated in culture with progesterone developed large, convoluted glands with prominent subnuclear vacuolizations (Fig. 2, *B* compared to *D*). No loss of tissue integrity was observed in endometrial explants after 24 h of culture with either control tissue or steroid treatment groups.

Establishment of human endometrial lesions in nude mice

The above experiments confirmed that short-term steroidal treatment of human endometrium in an explant culture system was sufficient both to initiate a morphological response, and to modulate secretion of stromal and epithelial-specific MMPs. Subsequently, we needed to determine whether short-term cultures of endometrial tissue compromised the ability of the human tissue to establish viable ectopic lesions in nude mice. A set of initial experiments, designed to promote endometrial MMP secretion, was performed using tissues first maintained in vitro in the presence of estradiol, and subsequently injected into nude mice with estradiol-releasing pellets. Confirming the ability of endometrial tissue to establish ectopic lesions after a period of in vitro culture, 11 of 12 nude mice receiving endometrial tissue maintained in a continuous estradiol environment developed peritoneal lesions. Gross visual examination of lesions before removal from the peritoneum of recipient mice revealed endometriotic-like tissue with a white or reddish appearance typical of the early human disease of endometriosis (Fig. 3, *A–D*). In addition to similarity in gross appearance, histologic sections of the ectopic endometrial lesions appear similar to lesions removed from women with the native disease. After staining with hematoxylin and eosin, lesions exhibit a distinctive glandular epithelium surrounded by an intact basement membrane and adjacent stromal cell layer of variable thickness and organization (Fig. 3, *E–F*).

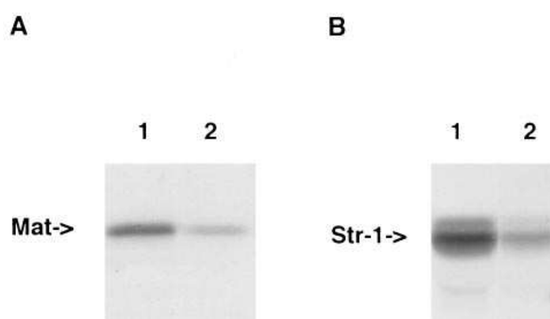

Figure 1. Steroid regulation of MMP expression in vitro. Detection and steroidal regulation of (*A*) promatrilysin (*Mat*), and (*B*) prostromelysin-1 (*Str-1*) protein expression in explants of proliferative phase human endometrium maintained in culture for 24 h with steroid. Lane *1*, 10 nM estradiol; lane *2*, 1 nM estradiol + 500 nM progesterone. (Results shown are representative; $n = 5$.)

Steroidal effects on establishment of endometriotic-like lesions

The preliminary experiments described above determined that human endometrial tissue, after a 24-h preincubation, retains the ability to establish endometriotic-like lesions in the peritoneal cavity of nude mice. We subsequently tested whether steroid treatment of endometrial tissue in vitro versus recipient animals in vivo differentially affects the ability of human tissue to establish ectopic lesions in the nude mouse model of endometriosis.

Estradiol effects. As summarized in Table I, estradiol treatment of endometrial tissues in vitro was found to be the most critical factor in determining whether or not endometriotic-like lesions were established in recipient animals. Treatment of endometrial tissue in vitro with estradiol resulted in the establishment of ectopic lesions in 100 or 90% of recipient mice, depending on whether the animals had also received subcutaneous estradiol-releasing pellets before injection of human tissue (Table I). In contrast, significantly fewer animals developed ectopic lesions in the absence of estradiol treatment of endometrial tissue in vitro. Only 30% of recipient animals with an estradiol-releasing pellet developed endometriotic-like lesions when receiving estradiol-deprived human tissue, while only 15% of animals developed lesions in the absence of steroid treatment of either the human tissue or the recipient animal.

Progesterone effects. In contrast to the positive effect of estradiol on the establishment of experimental endometriosis, progesterone treatment of endometrial tissues essentially blocked lesion formation in recipient mice regardless of the

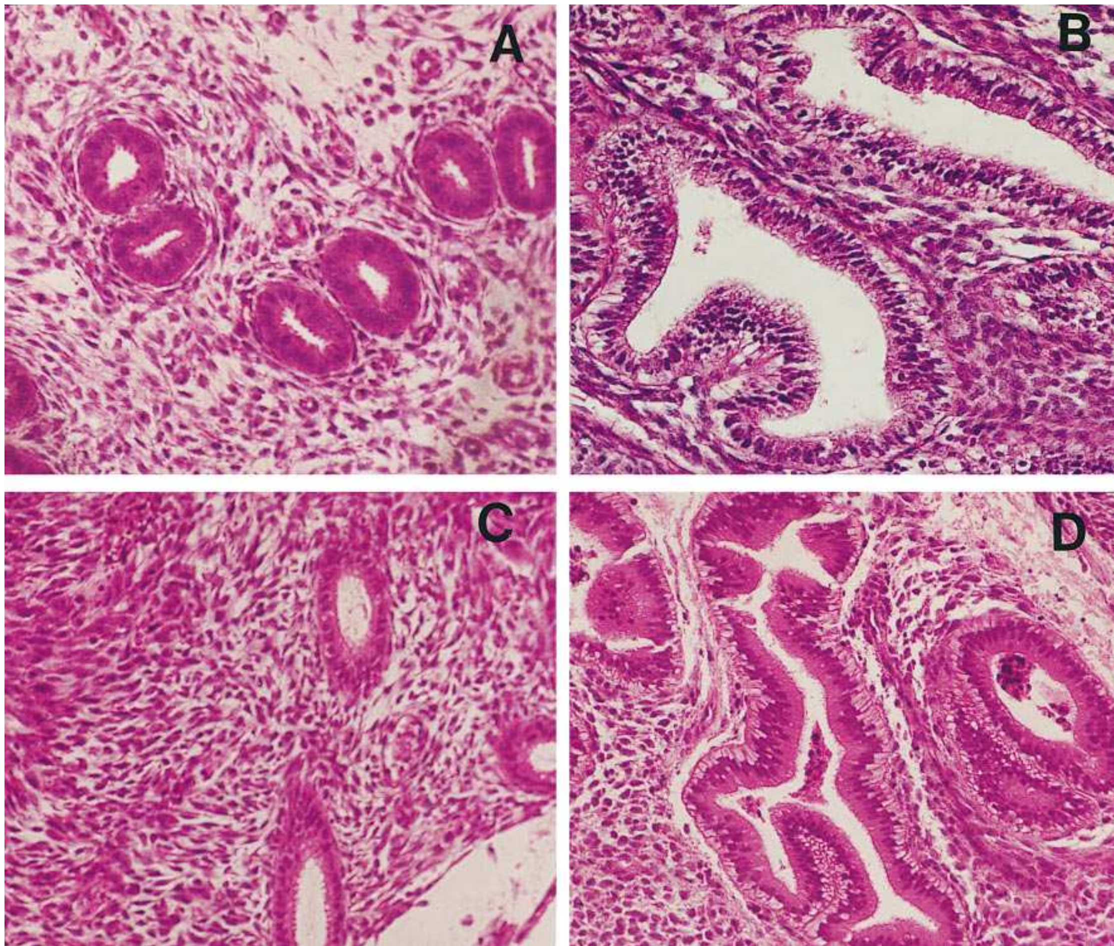

Figure 2. Steroid regulation of endometrial morphology. Formalin-fixed, paraffin-embedded proliferative phase human endometrium cultured in vitro for 24 h with estradiol or estradiol and progesterone were stained with hematoxylin and eosin. (*A*) Treatment with 10 nM estradiol results in the small, simple glands associated with the estrogen-dominated proliferative phase. (*B*) Tissue cultured with 1 nM estradiol and 500 nM progesterone develops prominent subnuclear vacuoles that identify the early secretory phase in vivo. Hematoxylin and eosin stain of normal endometrium demonstrates the typical morphology of midproliferative phase (*C*) or early secretory phase (*D*) tissue. ×200. (Results shown are representative; $n = 5$.)

steroid treatment of the animal (Table I). Reflecting the potent inhibitory effect of progesterone in this model, only 40% of mice implanted with progesterone-secreting pellets developed endometriotic-like lesions, even when receiving tissue pretreated with estradiol in vitro. Increasing estradiol exposure of tissue in vitro to 10 nM did not overcome the inhibitory effect of progesterone treatment on lesion formation (results not shown).

TIMP-1 effects on establishment of endometriotic-like lesions

The above experiments confirmed that steroids can act either to promote or prevent establishment of endometriotic-like lesions by human tissue in nude mice. While MMP secretion is regulated by steroid treatments, however, these experiments did not address directly the action of these enzymes on establishment of ectopic lesions. To examine more directly the role of secreted MMPs in the establishment of endometriotic-like lesions in our experimental model, an inhibitor of MMP activity, TIMP-1, was used to block the action of these enzymes. Since estradiol treatment of endometrial tissue and recipient animals resulted in the highest overall incidence of ectopic lesion formation, TIMP-1 experiments were conducted in concert with estradiol treatments. TIMP-1 treatments in vitro followed by peritoneal injections of TIMP-1 reduced the development of ectopic lesions to the highest level of significance ($P \leq 0.001$) compared to estradiol treatment alone (100% of animals developed lesions; Table II). The ability of TIMP-1 treatments to block ectopic lesion formation was reduced, but remained significant ($P \leq 0.01$), if the inhibitor was given only during the in vitro tissue incubation period (50% of animals developed lesions), or given only via peritoneal injections to the recipient mice in vivo (37% of animals developed lesions).

Discussion

The steroid sensitivity of the human endometrium is critical to the normal function of this specialized tissue, and the endometrium provides an excellent model system to examine the mechanisms by which steroids control specific cellular behav-

Table I. Effects of Steroid Treatment of Endometrial Tissue In Vitro on the Subsequent Development of Endometriotic-like Lesions in Nude Mice

Tissue treatment	Animal treatment	No. with lesions	Total No. of animals	Avg. No. of lesions in diseased mice	% with lesions
E2	E2	11	11	2.7	100*
E2	Control	9	10	1.4	90*
Control	E2	3	10	1.3	30‡
Control	Control	3	20	1.0	15
E2 and P4	E2	0	10		0§
E2	P4	4	10	1.5	40‖

Explants of human endometrium were obtained during the proliferative phase of the menstrual cycle and cultured in vitro for 24 h in 10 nM estradiol (E2), or 1 nM estradiol and 500 nM progesterone (E2 and P4), or no steroid (control). Tissues treated in vitro were subsequently injected into ovariectomized nude mice that had received a subcutaneous pellet containing estradiol (E2), progesterone (P4), or no steroid (control). *Avg*., average. *$P \leq 0.01$ when compared to control mice receiving control tissue. ‡P was not statistically significant when compared to control mice receiving control tissue. §$P \leq 0.005$ when compared to mice receiving E2 pellets and E2-treated tissue. ‖$P \leq 0.01$ when compared to mice receiving E2 pellets and E2-treated tissue.

Table II. Effects of TIMP-1 on the Establishment of Endometriotic-like Lesions in Nude Mice

Tissue treatment	Animal treatment	No. with lesions	Total No. of animals	Avg. No. of lesions in diseased mice	% with lesions
E2	E2	8	8	2.5	100
E2	E2 + TIMP-1	3	8	1.6	37.5*
E2 + TIMP-1	E2	4	8	1.25	50*
E2 + TIMP-1	E2 + TIMP-1	1	10	1.0	10‡

Explants of human endometrium were obtained during the proliferative phase of the menstrual cycle and cultured in vitro for 24 h in 10 nM estradiol (E2) or E2 with 0.2 μg TIMP-1. Tissues treated in vitro were subsequently injected into ovariectomized nude mice that had received a subcutaneous pellet containing estradiol (E2). Some animals also were given intraperitoneal TIMP-1 injections at 0, 4, 8, and 12 h after tissue injection (E2 + TIMP-1). *Avg*., average. *$P \leq 0.01$ when compared to mice receiving E2 pellets and E2-treated tissue. ‡$P \leq 0.001$ when compared to mice receiving E2 pellets and E2-treated tissue.

ior. For example, studies from several laboratories have begun to use the human endometrium to examine both direct and indirect mechanisms by which steroids can regulate MMP expression (7, 8, 10). The MMPs are important mediators of extracellular matrix remodeling in the female reproductive tract, and the cyclic expression of members of the stromelysin family in the endometrium reflects the dynamic nature of the human menstrual cycle (5, 6). The normal endometrium provides a unique view of steroid-sensitive MMP expression, but the role these biologically potent enzymes may play in establishing ectopic sites of endometrial tissue growth has not been previously examined. Endometriosis can arise from tissue shed during menstrual breakdown of the eutopic endometrium (11, 12), and can mimic malignant progression in that new ectopic lesions can be established via colonization of existing lesions (24). Ectopic lesions retain, to a variable degree, the steroid sensitivity of the highly specialized endometrium, and endometriosis often regresses in women in the progesterone-rich environment of pregnancy (25) or during progestin therapy (26). Estrogen exposure, on the other hand, may predispose women and nonhuman primates to the disease. Many of the risk factors for endometriosis, such as early menarche and nulliparity, are associated with prolonged estrogen exposure (18). Recent studies indicate that endometriotic lesions may be capable of producing estrogen, which may also contribute to the pathophysiology of this disease (27).

While the association of steroids with the development, recurrence, or remission of endometriosis has been recognized for some time, the cellular mechanisms by which estrogen and progesterone may affect the establishment of this disease, including a specific role for MMPs, remain speculative. Early human studies documented that direct introduction of endometrium into the peritoneal cavity of women can result in the establishment of endometriosis (13, 14). Direct studies in humans clearly are no longer appropriate. Therefore, we have used the nude mouse as a recipient animal for human tissue to examine the role steroid-sensitive MMP expression might play in the establishment of endometriosis. Several investigators have documented the utility of using nude mice for the ectopic growth of both normal human endometrium and endometriotic tissue (21, 28), and SCID mice recently have been used to establish a model of endometriosis (29). In the study reported here, we extend earlier observations by redirecting the biochemical phenotype and pattern of MMP secretion in endometrial tissue in vitro before peritoneal injection into recipient animals. We find that steroidal treatment of human endometrial tissue in vitro can redirect cellular phenotype and the secretion pattern of MMPs of the stromelysin family. Tissue treated with progesterone rapidly lost the ability to secrete MMPs in vitro, and subsequently failed to establish ectopic lesions when injected into nude mice. Conversely, the continued secretion of MMPs in organ cultures given estradiol alone enhanced the ability of the tissue to establish ectopic lesions in recipient mice. Importantly, blocking the action of secreted MMPs after estradiol pretreatment with a specific MMP inhibitor, TIMP-1 significantly inhibited the establishment of lesions in recipient mice. Together, these results indicate a direct role for MMPs in the ability of human tissue to establish ectopic lesions within the peritoneum of recipient nude mice, and suggest that steroidal regulation of these enzymes is important in the pathophysiology of endometriosis. The relationship between MMP expression and the establishment of human endometriosis remains speculative, however, studies in a primate model have demonstrated recently the importance of proteolytic activity in the development of the disease (30).

In summary, the nude mouse is clearly a useful animal in which to study the mechanisms by which human endometrial tissue can establish viable lesions in the peritoneal cavity. Human tissue removed as ectopic lesions from nude mice appear identical morphologically and histologically to endometriotic lesions removed from women with the disease. Importantly, this study demonstrates a mechanistic link between the steroidal regulation of MMP secretion in human endometrial tissue and the establishment of an endometriotic-like disease in an

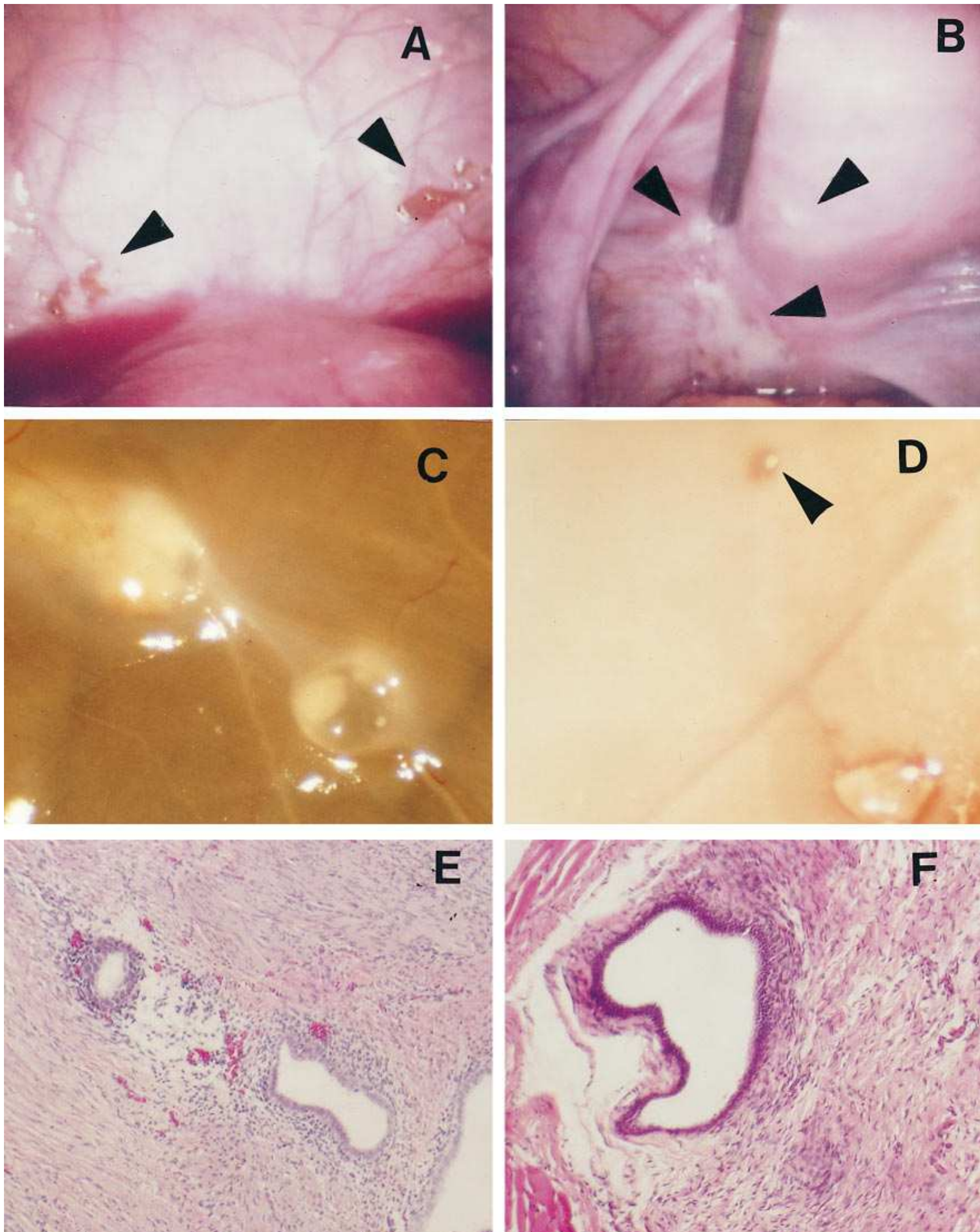

Figure 3. Endometriotic lesions: native disease vs. experimental model. Human lesions removed from nude mice are similar in appearance to the human disease on both gross and microscopic examination. Lesions of human endometriosis, photographed during a laparoscopic surgery performed during the proliferative phase of the menstrual cycle, can typically appear as small white or red nodules (*A* and *B*). Continuous estradiol treatments of human endometrium and nude mice result in ectopic lesions of similar appearance to the human disease (*C* and *D*). Formalin-fixed, paraffin-embedded human endometriotic lesions (*E*) or human tissues removed from mice as lesions (*F*) stained with hematoxylin and eosin again demonstrate marked similarities. ×200 for both photomicrographs.

experimental animal. Using this model system, future studies can analyze directly the relationship between steroids and other bioactive molecules that regulate MMP expression in endometrial tissue, and the invasive events necessary for the establishment or progression of the disease endometriosis.

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