0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 12
Printed in U.S.A.

Deficient 17β-Hydroxysteroid Dehydrogenase Type 2 Expression in Endometriosis: Failure to Metabolize 17β-Estradiol*

KHALED ZEITOUN, KAZUTO TAKAYAMA, HIRONOBU SASANO, TAKASHI SUZUKI, NABIL MOGHRABI, STEFAN ANDERSSON, ALAN JOHNS, LI MENG, MICHAEL PUTMAN, BRUCE CARR, AND SERDAR E. BULUN

Departments of Obstetrics-Gynecology (K.Z., K.T., N.M., S.A., A.J., L.M., M.P., B.C., S.E.B.) and Biochemistry (N.M., S.A.), University of Texas Southwestern Medical Center, Dallas, Texas 75235; and the Department of Pathology, Tohoku University School of Medicine (T.S.), Sendai, Japan

ABSTRACT

Aberrant aromatase expression in stromal cells of endometriosis gives rise to conversion of circulating androstenedione to estrone in this tissue, whereas aromatase expression is absent in the eutopic endometrium. In this study, we initially demonstrated by Northern blotting transcripts of the reductive 17β-hydroxysteroid dehydrogenase (17βHSD) type 1, which catalyzes the conversion of estrone to 17β-estradiol, in both eutopic endometrium and endometriosis. Thus, it follows that the product of the aromatase reaction, namely estrone, that is weakly estrogenic can be converted to the potent estrogen, 17β-estradiol, in endometriotic tissues.

It was previously demonstrated that progesterone stimulates the inactivation of 17β-estradiol through conversion to estrone in eutopic endometrial epithelial cells. Subsequently, 17βHSD type 2 was shown to catalyze this reaction, and its transcripts were detected in the epithelial cell component of the eutopic endometrium in secretory phase. Because 17β-estradiol plays a critical role in the development and growth of endometriosis, we studied 17βHSD-2 expression in endometriotic tissues and eutopic endometrium. We demonstrated, by Northern blotting, 17βHSD-2 messenger ribonucleic acid (RNA) in all RNA samples of secretory eutopic endometrium (n = 12) but not in secretory samples of endometriotic lesions (n = 10), including paired samples of endometrium and endometriosis obtained simultaneously from eight patients. This messenger RNA was not detectable in any samples of proliferative eutopic endometrium or endometriosis (n = 4) as expected. Next, we confirmed these findings by demonstration of immunoreactive 17βHSD-2 in epithelial cells of secretory eutopic endometrium in 11 of 13 samples employing a monoclonal antibody against 17βHSD-2, whereas 17βHSD-2 was absent in paired secretory endometriotic tissues (n = 4). Proliferative eutopic endometrial (n = 8) and endometriotic (n = 4) tissues were both negative for immunoreactive 17βHSD-2, except for barely detectable levels in 1 eutopic endometrial sample. Finally, we sought to determine whether deficient 17βHSD-2 expression in endometriotic tissues is due to impaired progesterone action in endometriosis. We determined by immunohistochemistry the expression of progesterone and estrogen receptors in these paired samples of secretory (n = 4) and proliferative (n = 4) eutopic endometrium and endometriosis, and no differences could be demonstrated. In conclusion, inactivation of 17β-estradiol is impaired in endometriotic tissues due to deficient expression of 17βHSD-2, which is normally expressed in eutopic endometrium in response to progesterone. The lack of 17βHSD-2 expression in endometriosis is not due to alterations in the levels of immunoreactive progesterone or estrogen receptors in this tissue and may be related to an inhibitory aberration in the signaling pathway that regulates 17βHSD-2 expression. (*J Clin Endocrinol Metab* **83:** 4474–4480, 1998)

ENDOMETRIOSIS is a common disorder in reproductive-aged women that has been linked to pelvic pain and infertility (1, 2). The most widely accepted theory for the development of pelvic endometriosis is implantation of viable endometrium on peritoneal surfaces after retrograde menstruation, as proposed by Sampson (3). Multiple genetic and environmental factors seem to be important in the etiology of endometriosis (4). Two proposed mechanisms are 1) deficiencies in the immune system that impair the clearance of the pelvic peritoneum of retrograde menstrual debris, and 2) intrinsic molecular aberrations in endometriotic implants (in comparison with the eutopic endometrium) that favor the implantation process. Biochemical differences between endometriotic tissues and the eutopic endometrium were reported by several investigators (5–10). Additionally, an increasing body of evidence points to differences between eutopic endometrium of women with endometriosis and that of disease-free women (5, 6, 11, 12).

Both circumstantial and laboratory evidence indicate that endometriosis is an estrogen-dependent disease. The formation of estrogens from C_{19} steroids is catalyzed by aromatase P450, which is expressed in significant levels in endometriotic tissues and stromal cells in culture, but not in the eutopic endometrium (5, 10, 13, 14). This was suggestive of a role of estrogen formed *in situ* in addition to circulating estrogens in the development and growth of endometriotic implants. The primary C_{19} substrate for aromatase in extraglandular tissues such as adipose and endometriotic tissues is circulating androstenedione that is converted to estrone. Estrone itself, however, is only weakly estrogenic and must be reduced by

Received June 2, 1998. Revision received August 27, 1998. Accepted August 28, 1998.

Address all correspondence and requests for reprints to: Serdar E. Bulun, M.D., Department of Obstetrics-Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235-9051. E-mail: bulun@grnctr.swmed.edu.

* This work was supported in part by an unrestricted research grant from American Society for Reproductive Medicine-Organon (to S.E.B.), NIH Grants R01-DK-52167 (to S.A. and N.M.) and 5-T32-HD-07190 (to N.M.), and an American Association of Obstetricians and Gynecologists Foundation Postdoctoral Fellowship Award (to K.Z.).

17β-hydroxysteroid dehydrogenase (17βHSD) type 1 to the potent estrogen, 17β-estradiol, for attainment of full estrogenic effect (15, 16). Another 17βHSD isozyme, 17βHSD type 2, catalyzes the conversions of 17β-estradiol to estrone and testosterone to androstenedione in a number of human tissues, including the placenta and liver (17). Additionally, very high levels of 17βHSD type 2 messenger ribonucleic acid (mRNA) have been demonstrated in the glandular epithelial cell fraction of the human endometrium during the secretory phase, suggesting that progesterone stimulates this enzyme (18, 19). In fact, estradiol dehydrogenase activity (oxidation of 17β-estradiol to estrone) in the endometrium has been shown to be stimulated by progesterone in earlier reports (20–22). The inactivation of 17β-estradiol to estrone by the secretory phase endometrium has been viewed as an important protective mechanism in this estrogen-responsive tissue. The expression of 17βHSD type 2 protein in the eutopic endometrium, however, has not been demonstrated to date. Additionally, the presence or absence of any type of 17βHSD expression in endometriotic tissues has not been investigated.

It should be noted that a third 17βHSD isozyme (type 3) catalyzes the reduction of androstenedione to testosterone in the microsomes of testicular Leydig cells (23, 24). Type 3 isozyme can also catalyze the reduction of estrone to 17β-estradiol. The mRNAs for these three proteins with aforementioned 17βHSD enzyme activities are encoded by three separate genes (16–18, 24). Because they play vital roles in 17β-estradiol biosynthesis or metabolism, we studied herein endometriotic and eutopic endometrial tissues to address the following questions. 1) As aberrant aromatase expression in endometriotic stromal cells gives rise to formation of primarily estrone, is there a 17βHSD isozyme in endometriosis that can catalyze the conversion of estrone to 17β-estradiol? 2) Is the protective mechanism of 17β-estradiol inactivation by 17βHSD type 2 in the secretory eutopic endometrium impaired in endometriotic tissues to give rise to increased local 17β-estradiol levels? Thus, we determined the transcript and protein levels of 17βHSD isozymes using RT-PCR, Northern blotting, and immunohistochemistry in the eutopic endometrium and endometriosis. We also report, for the first time in the literature, the localization of 17βHSD type 2 protein to secretory eutopic endometrial glandular cells.

Materials and Methods

Tissue acquisition and processing, and RNA isolation

At the time of laparoscopy or laparotomy the following types of samples were obtained: 1) extraovarian endometriotic implants from patients with endometriosis, 2) eutopic endometrial tissues from patients with endometriosis, 3) eutopic endometrial tissues from disease-free patients, 4) normal peritoneum from patients with endometriosis. Patients were between 24–38 yr of age with regular cycles. All specimens were confirmed histologically, and the phase of the cycle was determined by histological examination of the endometrium. Tissue samples were frozen instantly in liquid nitrogen in the operating room and stored at −80 C. Total RNA was isolated from tissues by the guanidium thiocyanate-cesium chloride method (25). Samples for immunohistochemistry were transported in 10% formalin and embedded in paraffin. Written informed consent was obtained before surgical procedures, including a consent form, and the protocol was approved by the institutional review board for human research of the University of Texas Southwestern Medical Center.

RT-PCR/Southern Hybridization

RT-PCR amplification of sequences in the coding regions of 17βHSD type 1, 2, and 3 mRNA species was performed using specific oligonucleotides (Table 1). The oligonucleotide primers were designed to flank at least three exons in each complementary DNA (cDNA; Table 1). This procedure involved synthesizing initially a cDNA by reverse transcription of 5 μg total RNA using Superscript II reverse transcriptase (BRL, Gaithersburg, MD) and random primers. Specific regions in the coding sequences of 17βHSD-1, -2, and -3 cDNAs were then amplified by PCR. The reaction was carried out in a 50-μl volume using Amplitaq DNA polymerase (Perkin Elmer/Cetus, Norwalk, CT), 1 mmol/L deoxy (d)-NTPs, 10 μmol/L sense and antisense primers, and PCR buffer (Perkin Elmer/Cetus) for 35 amplification cycles. Denaturing was performed at 94 C for 40 s, annealing at 58 C for 40 s, and extension at 72 C for 40 s. The generated PCR products were then size-fractionated on 1.8% agarose gel and transferred to nylon membranes for Southern blot analysis. The membranes were hybridized for 16 h at 42 C using specific cDNA probes radiolabeled with [α-^{32}P]dCTP. They were then exposed to x-ray film at −80 C for varying lengths of time.

Northern blotting

Total RNA (20 μg/lane) was size-fractionated by electrophoresis on 1% formaldehyde-agarose gels and transferred electrophoretically to a nylon membrane. The RNA was cross-linked to the nylon membranes by UV irradiation. The membranes were prehybridized for 24 h at 42 C in prehybridization buffer comprised of formamide (50%, vol/vol), NaH_2PO_4 (250 mmol/L; pH 7.2), NaCl (250 mmol/L), SDS (7%, wt/vol), and denatured sheared salmon sperm DNA (100 μg/mL). Hybridizations were conducted for 16 h at 42 C in the same buffer by adding 17βHSD type 1, 17βHSD type 2, or 17βHSD type 3 full-length cDNA probes (2×10^6 cpm/mL) radiolabeled with [α-^{32}P]dCTP using random hexanucleotide primers and Klenow. After hybridization, the blots were washed with varying concentrations of SSC (standard saline citrate) and SDS at varying temperatures. The membranes were exposed to film with intensifying screens at −80 C for varying lengths of time. The presence of comparable amounts of total RNA in each lane was verified by visualization of 18S and 28S ribosomal RNA subunits or by hybridization of membranes with a β-actin cDNA probe (Clontech Laboratories, Inc., Palo Alto, CA).

Immunohistochemistry

Immunohistochemistry for 17βHSD type 2, estradiol receptors (ER), and progesterone receptors (PR) in eutopic endometrial and endometriotic tissues was performed using monoclonal antibodies. The monoclonal antibody, mAb-C2-12, against 17βHSD type 2 was produced as previously described (26). Monoclonal antibodies against ERα (ERIDS) and PR (both A and B, MAB429) were purchased from Immunotech (Marseille, France) and Chemiron International, Inc. (Temecula, CA), respectively. The immunohistochemical procedures were performed as previously described on 2.5-μm-thick sections mounted on silane-coated slides using the biotin-streptavidin-amplified technique with a Histofine immunostaining system (Nichirei, Tokyo, Japan). Briefly, the staining procedure was performed as follows: 1) routine deparaffinization; 2)

TABLE 1. Sequence of oligonucleotides used as primers for PCR of 17βHSD isozyme transcripts

Isozymes	Sense primer	Antisense primer
17βHSD type 1	5′-AGGCTTATGCGAGAGTCTGG-3′ (exon 4)	5′-CTAGGCGGTGACGTAGTTGG-3′ (exon 6)
17βHSD type 2	5′-CTGAGGAATTGCGAAGAACC-3′ (exon 2)	5′-GAAGTCCTTGCTGGCTAACG-3′ (exon 7)
17βHSD type 3	5′-ACAATGTCGGAATGCTTCC-3′ (exon 5)	5′-AGGTTGAAGTGCTGGTCTGC-3′ (exon 11)

inactivation of endogenous peroxidase with 0.3% H_2O_2 in methyl alcohol for 20 min at 23 C; 3) blocking with 1% goat serum for 20 min at 23 C; 4) incubation with the primary antibody at 4 C for 18 h; 5) incubation with biotinylated goat antirabbit antibody for 20 min at 23 C; 6) incubation with peroxidase-conjugated streptavidin for 20 min at 23 C; 7) immersion in a solution containing 0.05% Tris-HCl (pH 7.6), 0.001 mol/L 3.3′-diaminobenzidine, and 0.006% H_2O_2; and 8) counterstaining with hematoxylin. For negative controls, normal mouse IgG was used instead of the primary antibodies. As positive controls, tissue sections of ER- and PR-positive breast cancer were used for ER and PR, and those of placenta were used for 17βHSD type 2 (27–29).

The dilutions of the primary antibodies are as follows: ER, 1:1; PR, 1:50; and 17βHSD type 2, 1:5. The dilution of the secondary antibody was 1:1. The semiquantification of ER and PR immunoreactivity was performed using labeling index as previously described (29).

Results

Detection of mRNAs of 17βHSD types 1, 2, and 3 in eutopic endometrial and endometriotic tissues by RT-PCR

Initially using RT-PCR, mRNAs for 17βHSD types 1, 2, and 3 were detected in the majority of extraovarian endometriotic and eutopic endometrial tissue samples (data not shown). Type 1 17βHSD mRNA was detected in all eutopic endometrial (n = 6) and endometriotic (n = 10) tissue samples. Type 2 17βHSD mRNA was detected in all 8 eutopic endometrial and in 7 of 8 endometriotic tissues. Type 3 17βHSD mRNA was detected in 30 of 33 eutopic endometrial samples and in all endometriotic samples (n = 10). Both proliferative and secretory endometriotic and eutopic endometrial tissues were included in this study, and no differences were noted between tissues in various phases of the cycle. Because RT-PCR is sufficiently sensitive to detect physiologically insignificant quantities of mRNA, we decided to use Northern blotting to demonstrate the presence or absence of significant quantities of mRNA.

Detection of mRNAs of 17βHSD types 1, 2, and 3 in eutopic endometrial and endometriotic tissues by Northern analysis

Type 1 17βHSD mRNA was detected by Northern analysis in both eutopic endometrial (12 secretory and 4 proliferative) and endometriotic (10 secretory and 4 proliferative) tissues after exposure of the film for 80 h at −80 C (Figs. 1 and 2). There were no apparent differences in the steady state levels of 17βHSD type 1 mRNA between eutopic endometrial and endometriotic samples in different cycle phases.

Type 2 17βHSD mRNA was detected by Northern blotting in all secretory phase eutopic endometrial samples (n = 12), whereas this mRNA was not detected in any of the proliferative eutopic endometrial samples (n = 4), as expected. In none of the secretory (n = 10) or proliferative (n = 4) endometriotic tissues, on the other hand, was 17βHSD type 2 mRNA detectable. Representative experiments are shown in Figs. 1 and 2. Included among these samples were eight pairs of eutopic endometrial and endometriotic tissues that were simultaneously acquired from eight patients undergoing laparoscopy during the secretory phase. All secretory eutopic endometrial samples (n = 8) contained 17βHSD type 2 mRNA, whereas all simultaneously biopsied endometriotic tissues were negative (n = 8) for 17βHSD type 2 mRNA using Northern blotting (Figs. 1 and 2). Type 3 17βHSD mRNA was not detected by Northern analysis in any of the eutopic endometrial (n = 16) or endometriotic (n = 14) tissues even after prolonged exposure (Fig. 1). Normal-appearing peritoneal samples from patients with endometriosis (n = 6) biopsied during laparoscopy were negative for 17βHSD type 2 by Northern analysis (Fig. 1). No differences were detected between eutopic endometrial samples from patients with endometriosis and those from disease-free women.

Immunohistochemistry for 17βHSD type 2

Type 2 17βHSD was detected in eutopic endometrial glandular epithelial cells in 11 of 13 secretory phase eutopic endometrial samples. One of the negative endometrial samples was in the very early secretory phase, and the other had evidence of chronic endometritis. Immunoreactive 17βHSD type 2 became detectable in the early secretory phase and increased during the midsecretory phase, and the intensity of staining was the highest in the late secretory phase. Immunoreactive 17βHSD type 2 was detected in only 1 of 8 proliferative eutopic endometrial samples. In this 1 sample,

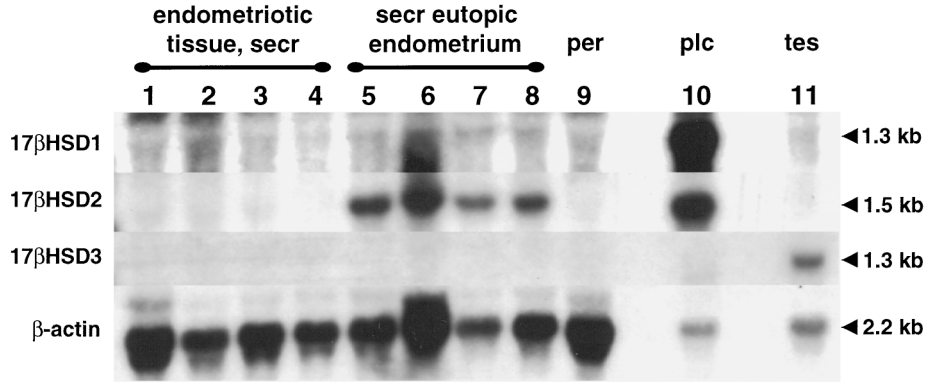

FIG. 1. Northern blot analysis of total RNA (20 μg) from four secretory phase endometriotic (lanes 1–4) and four simultaneously biopsied (secretory phase) eutopic endometrial samples (lanes 5–8) and from normal peritoneum (per; lane 9). Three micrograms of total RNA from the following control tissues were used: placenta (plc; lane 10) and testis (tes; lane 11). Type 1 17βHSD mRNA was detected in both endometrial and endometriotic samples after prolonged exposure (80 h) of the blotted membrane (positive control: placenta). The mRNA of 17βHSD type 2 was detected only in the secretory phase eutopic endometrium and placenta (positive control). Type 3 17βHSD mRNA was detected only in testicular RNA that was used as a positive control. The mRNA of β-actin was detected to check comparable loading of samples in different lanes.

however, intensity of immunostaining with 17βHSD type 2 antibody was very low. In addition to these, we also performed immunohistochemistry on simultaneously biopsied samples of eutopic endometrium and pelvic extraovarian endometriosis (n = 8). In these paired samples, no 17βHSD type 2 was detected in proliferative phase eutopic endometrial or endometriotic tissues (n = 4 pairs; Fig. 3). In secretory phase samples, however, 17βHSD type 2 was detected in the glandular cells of all eutopic endometrial specimens (n = 4), whereas the paired secretory phase endometriotic tissues were negative for immunoreactive 17βHSD type 2 (Fig. 3).

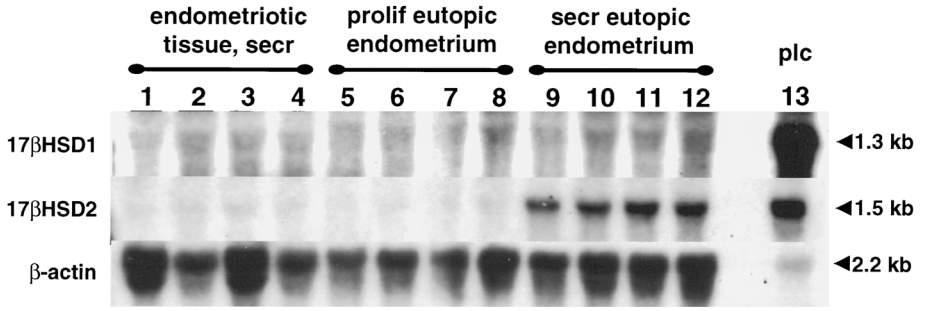

FIG. 2. Northern blot analysis of total RNA (20 μg) from four secretory phase endometriotic (lanes 1–4), four proliferative phase eutopic endometrial (lanes 5–8), and four secretory eutopic endometrial specimens (lanes 9–12). Placental RNA (plc; 3 μg; lane 13) was used as a positive control for 17βHSD types 1 and 2. mRNA of 17βHSD type 1 were detected in all eutopic endometrial and endometriotic samples after prolonged exposure. Type 2 17βHSD mRNA was detected only in secretory eutopic endometrial samples and the placenta. The lowest membrane demonstrates β-actin mRNA.

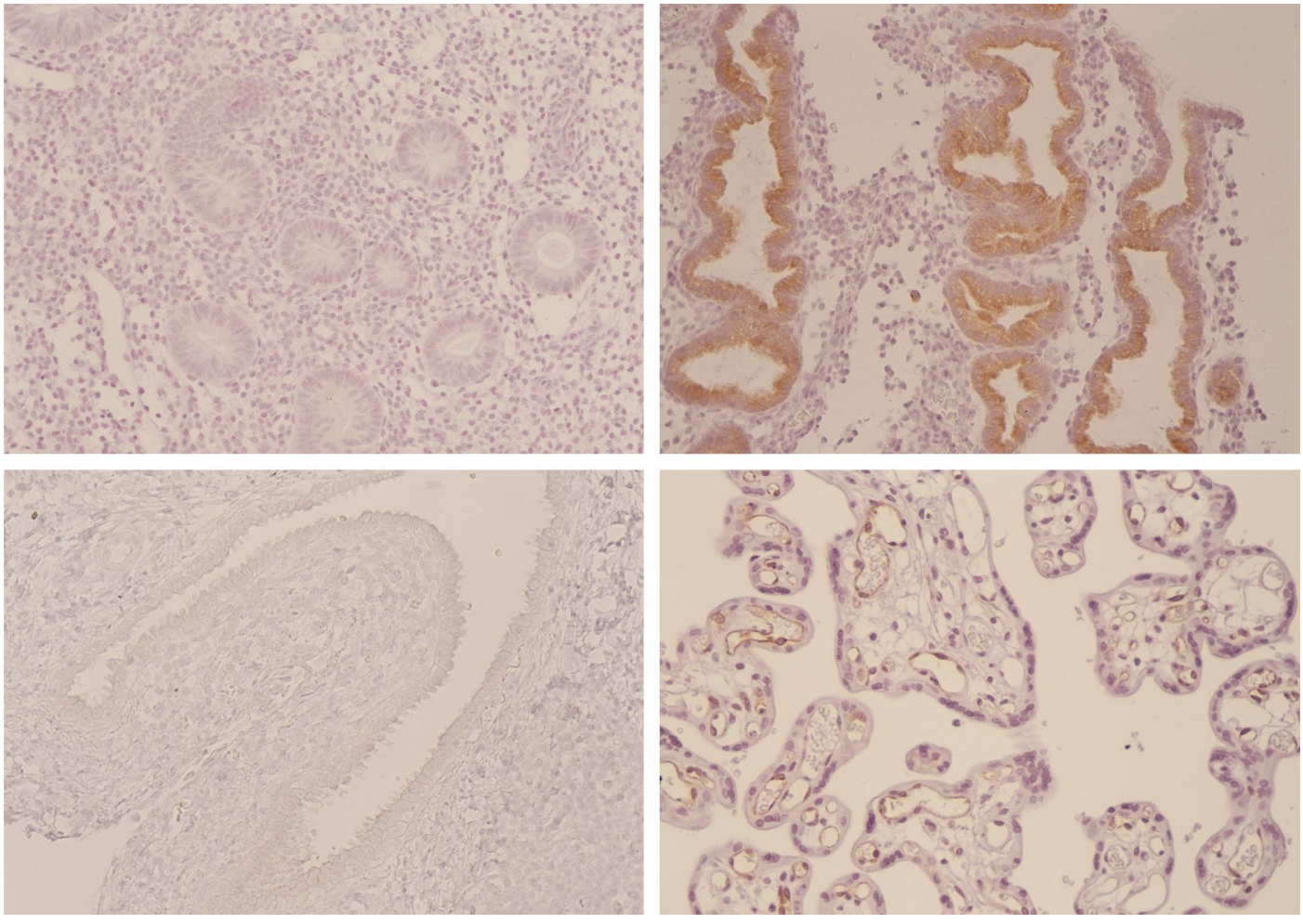

FIG. 3. Detection of immunoreactive 17βHSD type 2 using a monoclonal antibody (brown cytoplasmic stain). A, A section of proliferative eutopic endometrium with no evidence of staining. B, Intense staining of epithelial cells in the late secretory eutopic endometrium. C, Secretory endometriotic tissue biopsied simultaneously from the same patient at the time of eutopic endometrial sampling represented in B. No immunoreactive 17βHSD type 2 was noted. D, Placenta with positive staining of the vascular endothelial cells.

Immunohistochemistry for ER and PR

We hypothesized that deficient 17βHSD type 2 expression in the secretory endometriotic tissue may be due to deficient progesterone action, as progesterone was demonstrated to induce this enzyme in the eutopic endometrium (18, 21, 22). Thus, we determined immunoreactive PR and ER in simultaneously obtained paired samples (n = 8) of eutopic endometrium and endometriosis that were previously stained for 17βHSD type 2. Immunoreactive ER and PR were demonstrated in both glandular epithelial and stromal cells regardless of the cycle phase in all samples of eutopic endometrium and endometriosis (Fig. 4). No obvious differences in ER and PR expression were observed between eutopic endometrial and endometriotic tissues. Thus, it appears that differences in ER and PR expression do not account for the differential expression of 17βHSD type 2 expression in our samples.

Discussion

Type 2 17βHSD mRNA was previously demonstrated in epithelial cells of the eutopic endometrium during the secretory phase using cell fractionation and Northern blot analysis and also by *in situ* hybridization (18, 19). We demonstrated herein the localization of 17βHSD type 2 protein to secretory epithelial cells using a monoclonal antibody that we recently raised against 17βHSD type 2 (26).

We also sought to demonstrate the presence or absence of a reductive-type 17βHSD in the eutopic endometrium and endometriosis. To this end, we used RT-PCR and Northern analysis to detect mRNA species of two well defined reductive 17βHSDs, namely type 1 and type 3, both of which are capable of catalyzing the conversion of estrone to 17β-estradiol. RT-PCR gave rise to amplification of mRNAs for all three types of 17βHSDs in both tissues regardless of the cycle phase. As mRNA levels detected by PCR may not be physiologically relevant, we resorted to Northern blotting, which demonstrated 17βHSD type 1 mRNA in both eutopic endometrium and endometriosis during the proliferative and secretory phases. We concluded that the presence of a reductive enzyme, which catalyzes the conversion of locally produced estrone to the potent estrogen 17β-estradiol, might be an important pathophysiological complement to aberrant

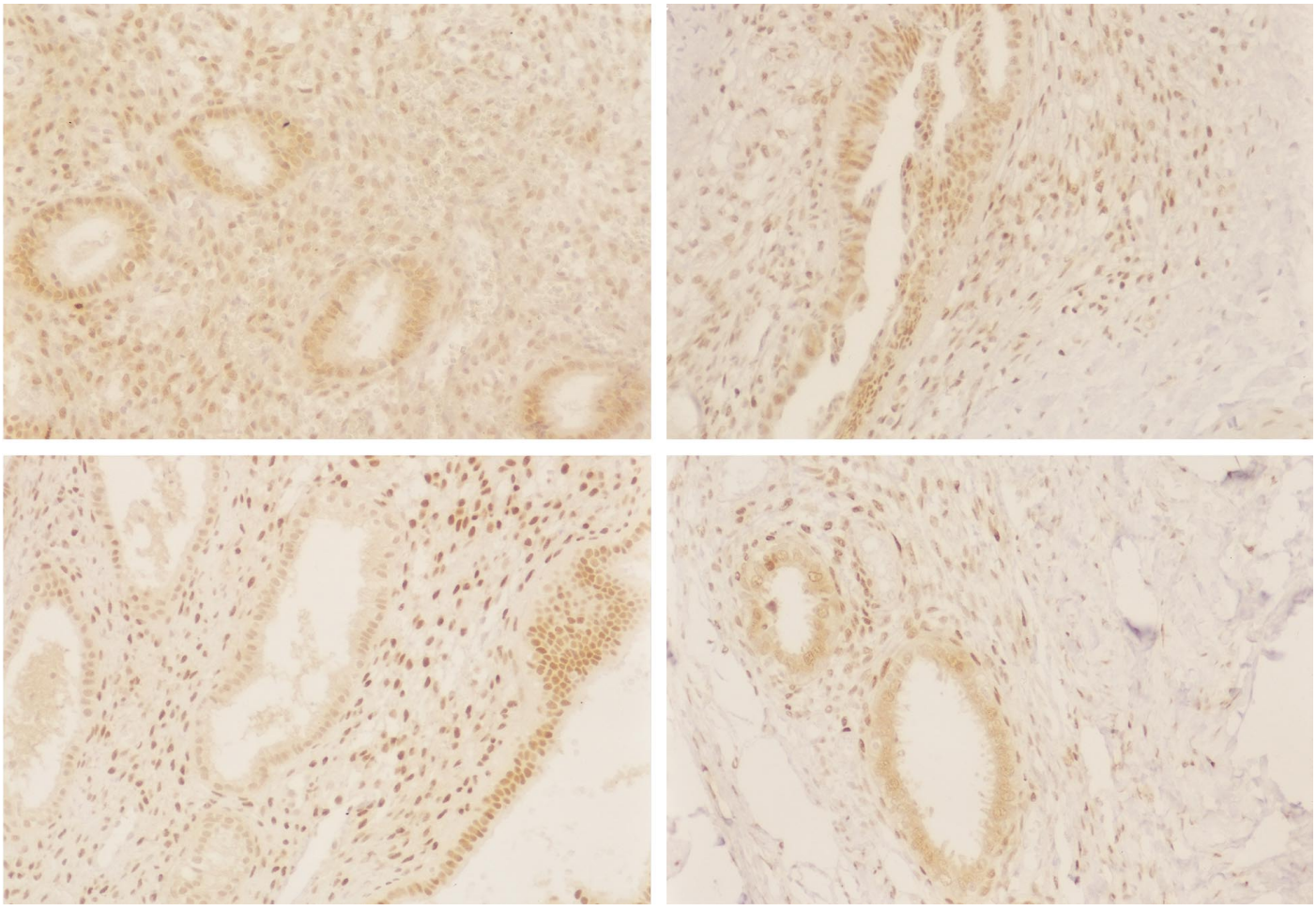

FIG. 4. Detection of immunoreactive PR (brown nuclear stain). A, A section of proliferative eutopic endometrium. B, A section of endometriosis in the proliferative phase (obtained from same patient represented in section A). C, Secretory endometrium. D, A section of endometriosis in the secretory phase (obtained from the same patient represented in section C). Both epithelial and stromal cell nuclei are immunoreactive for PR in all sections. There is evidence of thermal damage at the periphery of the endometriotic samples due to CO_2 laser used for excision.

aromatase expression in endometriosis for local estrogen production (5). The primary cell type that expresses 17βHSD type 1 in the eutopic endometrium or endometriosis is not known yet. Studies are under way to address this question.

The most exciting finding was the absence of progesterone-induced 17βHSD type 2 expression in epithelial cells of endometriotic tissues in contrast to those of the eutopic endometrium. This was demonstrated by Northern blotting and immunohistochemistry. Deficient expression of an enzyme that inactivates 17β-estradiol in endometriosis is supportive of a model in which a number of aberrations (*e.g.* aromatase expression) in this tissue serve to maintain maximal local concentrations of 17β-estradiol for prolonged periods.

The role of progesterone in the induction of 17βHSD type 2 in the eutopic endometrium is evident from previous studies on estradiol dehydrogenase activity and 17βHSD type 2 mRNA and protein expression, which were demonstrated only during the secretory phase (18–22). Thus, one may speculate that the absence of induction of 17βHSD type 2 in secretory endometriotic samples may result from defective progesterone exposure or action in endometriosis. For example, very low levels of progesterone reaching the endometriotic lesions because of poor blood supply may give rise to a relative progesterone deficiency. However, histological changes in response to progesterone are routinely noted in endometriotic lesions, indicative of at least a partial response to progesterone. Thus, poor circulation in endometriotic implants is not a likely cause of progesterone deficiency in endometriosis. Impaired progesterone action on endometriosis may also be explicable in terms of selective defects in the signaling pathway, such as the quantity of PRs. In fact, several investigators have previously reported decreased ER and PR contents in endometriotic glandular cells compared with those in the eutopic endometrium, whereas others found the opposite (30–36). Moreover, a group of investigators found that the ER and PR contents of endometriotic implants do not undergo predictable changes in response to endogenous hormones (11). To determine whether deficient 17βHSD type 2 expression in endometriotic epithelial cells is due to alterations in the ER/PR content, we localized these receptors by immunohistochemistry in simultaneously biopsied, paired samples of endometriosis and eutopic endometrium, which were previously used to study 17βHSD type 2 expression. No apparent differences in ER and PR expression between these two groups were observed. This, however, did not exclude defective receptors or altered ratios of PR subtypes in endometriosis. The absence or presence of a PR coregulator that gives rise to inhibition of progesterone action on selected target genes in endometriotic epithelial cells is another exciting possibility. We would like to point out, however, that the number of samples used was small, and immunohistochemistry is not a suitable quantitative method to compare small differences between tissues. In summary, 17βHSD type 2 deficiency in endometriotic tissues may give rise to higher local concentrations of 17β-estradiol. Because progesterone inhibits and estrogen stimulates the growth of endometriotic implants, partial progesterone resistance in endometriosis may be a key event that is responsible for the growth properties and molecular aberrations in this tissue. This does not, however, preclude the response of the tissue to pharmacological or continuous levels of progesterone during treatment of endometriosis with progestins.

Acknowledgment

Rosemary Bell provided skilled editorial assistance.

References

1. **Stovall DW, Bowser LM, Archer DF, Guzick DS.** 1997 Endometriosis-associated pelvic pain: evidence for an association between the stage of disease and a history of chronic pelvic pain. Fertil Steril. 68:13–18.
2. **Marcoux S, Maheux R, Bërubë S, Canadian Collaborative Group on Endometriosis.** 1997 Laparoscopic surgery in infertile women with minimal or mild endometriosis. N Engl J Med. 337:217–222.
3. **Sampson JA.** 1927 Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol. 14:422–425.
4. **Olive DL, Schwartz LB.** 1993 Endometriosis. N Engl J Med. 328:1759–1769.
5. **Noble LS, Simpson ER, Johns A, Bulun SE.** 1996 Aromatase expression in endometriosis. J Clin Endocrinol Metab. 81:174–179.
6. **Ryan I, Schriock ED, Taylor R.** 1994 Isolation, characterization, and comparison of human endometrial and endometriosis cells *in vitro*. J Clin Endocrinol Metab. 78:642–649.
7. **Sharpe KL, Zimmer RL, Griffin WT, Penney LL.** 1993 Polypeptides synthesized and released by human endometriosis differ from those of the uterine endometrium in cell and tissue explant culture. Fertil Steril. 60:839–851.
8. **Sharpe KL, Vernon MW.** 1993 Polypeptides synthesized and released by rat ectopic uterine implants differ from those of the uterus in culture. Biol Reprod. 48:1334–1340.
9. **Sharpe-Timms KL, Penney LL, Zimmer RL, Wright JA, Zhang Y, Surewicz K.** 1995 Partial purification and amino acid sequence analysis of endometriosis protein-II (ENDO-II) reveals homology with tissue inhibitor of metalloproteinases-1 (TIMP-1). J Clin Endocrinol Metab. 80:3784–3787.
10. **Noble LS, Takayama K, Putman JM, et al.** 1997 Prostaglandin E_2 stimulates aromatase expression in endometriosis-derived stromal cells. J Clin Endocrinol Metab. 82:600–606.
11. **Lessey BA, Metzger DA, Haney AF, McCarty Jr KS.** 1989 Immunohistochemical analysis of estrogen, and progesterone receptors in endometriosis: comparison with normal endometrium during the menstrual cycle and the effect of medical therapy. Fertil Steril. 51:409–415.
12. **Lessey BA, Castelbaum AJ, Sawin SW, et al.** 1994 Aberrant integrin expression in the endometrium of women with endometriosis. J Clin Endocrinol Metab. 79:643–649.
13. **Simpson ER, Zhao Y, Agarwal VR, et al.** 1997 Aromatase expression in health and disease. Recent Prog Horm Res. 52:185–214.
14. **Bulun SE, Noble LS, Takayama K, et al.** 1997 Endocrine disorders associated with inappropriately high aromatase expression. J Steroid Biochem Mol Biol. 61:133–139.
15. **Isomaa VV, Ghersevich SA, Mäentausta OK, Peltoketo EH, Poutanen MH, Vihko RK.** 1993 Steroid biosynthetic enzymes: 17β-hydroxysteroid dehydrogenase. Ann Med. 25:91–97.
16. **Labrie F, Luu-The V, Labrie C, et al.** 1989 Characterization of two mRNA species encoding human estradiol 17β-dehydrogenase and assignment of the gene to chromosome 17. J Steroid Biochem. 34:189–197.
17. **Wu L, Einstein M, Geissler WM, Chan HK, Elliston KO, Andersson S.** 1993 Expression cloning and characterization of human 17β-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20α-hydroxysteroid dehydrogenase activity. J Biol Chem. 268:12964–12969.
18. **Casey ML, MacDonald PC, Andersson S.** 1994 17β-Hydroxysteroid dehydrogenase type 2: chromosomal assignment and progestin regulation of gene expression in human endometrium. J Clin Invest. 94:2135–2141.
19. **Mustonen MVJ, Isomaa VV, Vaskivuo T, et al.** 1998 Human 17β-hydroxysteroid dehydrogenase type 2 messenger ribonucleic acid expression and localization in term placenta and in endometrium during the menstrual cycle. J Clin Endocrinol Metab. 83:1319–1324.
20. **Tseng L, Gurpide E.** 1974 Estradiol and 20α-dihydroprogesterone dehydrogenase activities in human endometrium during the menstrual cycle. Endocrinology. 94:419–423.
21. **Tseng L, Gurpide E.** 1975 Induction of human endometrial estradiol dehydrogenase by progestins. Endocrinology. 97:825–833.
22. **Satyaswaroop PG, Wartell DJ, Mortel R.** 1982 Distribution of progesterone receptor, estradiol dehydrogenase, and 20α-dihydroprogesterone dehydrogenase activities in human endometrial glands and stroma: progestin induction of steroid dehydrogenase activities *in vitro* is restricted to the glandular epithelium. Endocrinology. 111:743–749.
23. **Andersson S, Geissler WM, Wu L, et al.** 1996 Molecular genetics and pathophysiology of 17β-hydroxysteroid dehydrogenase 3 deficiency. J Clin Endocrinol Metab. 81:130–136.

24. **Andersson S, Moghrabi N.** 1997 Physiology and molecular genetics of 17β-hydroxysteroid dehydrogenases. Steroids. 62:143–147.
25. **Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ.** 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 18:5294–5299.
26. **Moghrabi N, Head JR, Andersson S.** 1997 Cell type-specific expression of 17β-hydroxysteroid dehydrogenase type 2 in human placenta and fetal liver. J Clin Endocrinol Metab. 82:3872–3878.
27. **Nakazumi H, Sasano H, Maehara I, Ozaki M, Tezuka F, Orikasa S.** 1996 Estrogen metabolism and impaired spermatogenesis in germ cell tumors of the testis. J Clin Endocrinol Metab. 81:1289–1295.
28. **Sasano H, Uzuki M, Sawai T, et al.** 1997 Aromatase in human bone tissue. J Bone Miner Res. 12:1416–1423.
29. **Sasano H, Frost AR, Saitoh R, et al.** 1996 Aromatase and 17β-hydroxysteroid dehydrogenase type 1 in human breast carcinoma. J Clin Endocrinol Metab. 81:4042–4046.
30. **Donnez J, Nisolle M, Smoes P, Gillet N, Beguin S, Casanas-Roux F.** 1996 Peritoneal endometriosis and "endometriotic" nodules of the rectovaginal septum are two different entitites. Fertil Steril. 66:362–368.
31. **Bergqvist A, Ferno M.** 1993 Oestrogen and progesterone receptors in endometriotic tissue and endometrium: comparison of different cycle phases and ages. Hum Reprod. 8:2211–2217.
32. **Bergqvist A, Ljungberg O, Skoog L.** 1993 Immunohistochemical analysis of oestrogen and progesterone receptors in endometriotic tissue and endometrium. Hum Reprod. 8:1915–1922.
33. **Prentice A, Randall BJ, Weddell A, et al.** 1992 Ovarian steroid receptor expression in endometriosis and in two potential parent epithelia: endometrium and peritoneal mesothelium. Hum Reprod. 7:1318–1325.
34. **Lyndrup J, Thorpe S, Glenthoj A, Obel E, Sele V.** 1987 Altered progesterone/estrogen receptor ratios in endometriosis. A comparative study of steroid receptors and morphology in endometriosis and endometrium. Acta Obstet Gynecol Scand. 66:625–629.
35. **Jones RK, Bulmer JN, Searle RF.** 1995 Immunohistochemical characterization of proliferation, oestrogen receptor and progesterone receptor expression in endometriosis: comparison of eutopic and ectopic endometrium with normal cycling endometrium. Hum Reprod. 10:3272–3279.
36. **Nisolle M, Casanas-Roux F, Wyns C, deMenten Y, Mathieu PE, Donnez J.** 1994 Immunohistochemical analysis of estrogen and progesterone receptors in endometrium and peritoneal endometriosis: a new quantitative method. Fertil Steril. 62:751–759.