0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 86, No. 3
Printed in U.S.A.

Presence of Estrogen Receptor β in the Human Endometrium through the Cycle: Expression in Glandular, Stromal, and Vascular Cells*

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ABSTRACT

The recent discovery of a new isoform of estrogen receptor (ER) β has prompted the reexamination of estrogen action on target organs. Here, we describe the endometrial expression of human ERβ and compare its distribution with that of ERα in the endometrial functional zone. Using immunocytochemistry with well characterized polyclonal antibodies against ERβ, we have detected specific ERβ expression in all endometrial compartments (glandular, stromal, and vascular); the specificity of the immunostaining is confirmed by lack of staining of the uterine sections with anti-ERβ antibodies previously incubated with peptide preparation. The highest levels of ERβ expression are observed in epithelial cells during the periovulatory period (days 14 and 15), as well as in stromal cells and cells of the vascular wall in the late-secretory phase; both smooth muscle cells and endothelial cells express ERβ, as deduced from immunocytochemistry and RT-PCR analysis. ERβ staining is usually low compared with that of ERα, except at days 24–26. The presence of ERβ in decidualized stromal cells is deduced from immunocytochemistry using antismooth α-actin and anti-ERβ antibodies or from RT-PCR analysis of ERβ and insulin-like growth factor-BP transcripts in the same cells; the presence of ERβ-positive stromal cells located close to vascular smooth muscle cells during this period suggests some specific role of this receptor during decidualization. ERα is also present in the cells of the endometrial vascular wall, in addition to the nuclei of glandular epithelial and stromal cells. Vascular ERα expression is highest during the periovulatory period, suggesting a regulation by estradiol, and a role in vascular function. Moreover, different variations of ERβ and ERα in arterioles might have implications for the modulation of vascular function, possibly of vascular tone, during the menstrual cycle. Finally, these data suggest that ERβ may have important roles in endometrial function, in addition to the well known role of ERα in endometrial proliferation and differentiation. (*J Clin Endocrinol Metab* **86,** 1379–1386, 2001)

ESTROGENS PLAY IMPORTANT roles in the regulation of cyclic changes in the human endometrium; these estrogenic effects were thought to operate via the estrogen receptor (ER) localized in epithelial, stromal, and vascular cells. Several studies have demonstrated that the levels of this receptor in epithelial cells vary during the different stages of the menstrual cycle (1, 2). Since the cloning of ER (3), it was generally accepted that only one receptor existed. However, the discovery of a new member of the nuclear receptor superfamily with specificity for estrogens (4–6) has prompted the reexamination of the estrogen signaling system. The novel receptor ERβ is highly homologous to the classical ER (ERα) and has been shown to stimulate transcription of an ER target gene (7). The most significant disparity between the two receptors lies in their tissue distribution; ERα messenger RNAs (mRNAs) are predominant in the uterus, mammary gland, testis, pituitary, liver, kidney, heart, and skeletal muscle, whereas ERβ mRNAs are significantly expressed in the ovary and prostate (8, 9). These studies lead to the conclusion that there is a low to absent expression of ERβ in the uterus, at least in the mouse (8), in agreement with some studies reporting insensitivity to estradiol (E_2), with respect to its effects on water imbibition, RNA and DNA synthesis, and mitosis of the glandular and stromal compartment in ERα knockout mice (αERKO) (10–11). However, the reported preservation of other selective estrogen actions in the αERKO uterus, such as induction of secretory products by the catecholestrogen 4-hydroxyestradiol (12), reinforces the need for studies about the exact endometrial distribution of the two distinct ERs.

Few studies have reported the presence of ERβ transcripts in the human endometrium (13, 14), whereas immunocytochemical detection in the uterine compartments has proven difficult in the past owing to the lack of available antibodies against ERβ. In the present study, we investigated the expression of ERβ in the human cyclic endometrium, using immunocytochemistry with previously characterized polyclonal anti-ERβ antibodies (15, 16), and determined the relative expression of ERα and ERβ in each cell compartment.

Materials and Methods

Human uterine tissues

Endometrial biopsies were obtained from 35 cycling women (age, 22–45 yr) undergoing routine gynecological investigations in Bari University Hospital in Italy. The selected patients had no evident endocrinological problems and had normal endometrial histology; none of the patients had taken hormonal medication for at least 6 months before surgery. Informed consent was obtained from each patient, and the

Received May 23, 2000. Revision received October 4, 2000. Rerevision received November 3, 2000. Accepted November 9, 2000.

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* Supported by the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, and the Association pour la Recherche sur le Cancer. Presented at the workshop "Molecular Genetics of Human Reproduction" from ARES-Serono Foundation, Heraklion, Crete, 2000.

study was approved by the local ethical commitee. Specimens of endometrium were obtained in the proliferative (n = 13), secretory (n = 18), and menstrual (n = 4) phases of the cycle. Endometrial dating criteria and independent histological examination were used to assess the phase of menstrual cycle (17). All tissues were fixed in 4% buffered paraformaldehyde and embedded in paraffin.

Immunocytochemistry

Paraffin sections of 5-μm thickness were routinely processed and mounted on Superfrost+ glass slides. Immunocytochemistry of E_2 receptor(s) was performed as described previously (17, 18), with the following modifications. Previous to immunocytochemical staining, antigen retrieval was performed on the paraffin sections by microwaving for 15 min in 0.1 M glycine-EDTA buffer (pH 3.15). The immunocytochemical staining included overnight incubation at 4 C with a mouse monoclonal antibody raised against the 67-kDa polypeptide chain of ERα (clone 1D5; Immunotech, Marseille, France) or affinity-purified sheep anti-ERβ receptor antibodies (a generous gift from P. Saunders; Ref. 15), followed by incubation with biotinylated antimouse or antisheep IgG and streptavidin-peroxidase (LSAB+ immunostaining kit; Dakopatts, Glostrup, Denmark). Peroxidase reaction was performed using aminoethylcarbazole substrate (Sigma, St. Louis, MO), as described previously (18). Some sections were lightly counterstained with Meyer's hematoxylin. Each immunoreaction was performed twice in triplicate. A commercially available goat anti-ERβ antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) was also used in some experiments with comparable results in ERβ distribution.

The following controls were performed: preincubation of anti-ERβ antibody with increasing amounts of purified recombinant peptide (a gift from P. Saunders; 1–100 μg/μL diluted antibody) for 12 h at 4 C before immunostaining; omission of the first antibody, and finally incubation of tissue sections with nonimmune mouse (DAKO Corp.) or sheep IgG (Serotec, Oxford, UK). Tissue sections of ERβ-positive prostatic adenoma and of an ERα-positive mammary adenocarcinoma were used as positive controls.

Adjacent sections were incubated with a marker of vascular endothelial cells, the polyclonal anti-Von Willebrand factor antibody (DAKO A/S, Glostrup, Denmark), or with a monoclonal antismooth α-actin antibody (Sigma) to identify smooth muscle cells of the vascular wall and decidualized stromal cells, as described previously (19, 20).

Evaluation of immunostaining and nuclear counting

Both staining intensity and number of stained cells in the endometrial functional zone were evaluated. The relative intensity of the immunoreaction product on the whole section was graded blindly by three independent observers (G.L., G.M., and M.P.-A.) using a light microscope Axiophot (Zeiss, Oberkochen, Germany) at a ×100 and ×200 magnification; it was estimated semiquantitatively on a scale of 0–3+ as: −, no detectable stain; −/+, faint; +, moderate; ++, strong; and +++, very intense staining, as described previously (18, 20, 21). To quantify the number of immunopositive cells, immunostained nuclei were counted separately in glandular, stromal, and vascular cells of the functional zone, using a microscope (Leitz, Orthoplan) equipped with a color CDD video camera. Five different fields in each section (×16 objective, 0.322 mm^2 per field) were digitized by image analysis and computerized using the Histolab program (Microvision, Evry, France), as described previously (18). Nuclear counts were assessed blindly for each section. Values were expressed as means/per unit area ± SEM. Measurements were tested by ANOVA with Fisher's test. Comparisons were made using the mean values for each biopsy; Student's t test was used to compare the ER levels of each group of cells in the proliferative phase with the levels seen in luteal phase. For all statistical analysis, P less than 0.05 was considered significant.

Isolation of human endometrial cells

Isolation of stromal cells from endometrial tissue was performed using dissociation with 0.1% collagenase and 0.02% DNAse I, as described previously (22, 23). Purity of the stromal cell preparation obtained at passage 3–4 was verified as described previously (22), by staining with anti-vimentin (clone V9; (DAKO Corp.) and antileucocyte common antigen (DAKO Corp.) antibodies. Cells were treated with epidermal growth factor (EGF; 20 ng/mL) plus E_2 (10^{-8} M) and progesterone (10^{-6} M) (23) for various lengths of time (1–14 days). Decidual cells were characterized by expression of PRL and insulin-like growth factor binding protein (IGFBP) (24) demonstrated by immunocytochemistry with specific antibodies (Eurodiagnostica, Upjohn Pharmacia).

Extraction of RNA from cells and reverse transcription (RT)-PCR analysis

Total RNA was isolated from cultures of stromal cells using a modified guanidium isothiocyanate method (Trizol; Life Technologies) (22). A semiquantitative RT-PCR method was established [as described previously (26) and modified (25)] to determine the amount of RNA and the number of cycles to be used in order that the quantity of PCR products is proportional to the quantity of total RNA and increases linearly as a function of the number of cycles. Amplification of ERβ complementary DNA (cDNA) was carried out in a DNA thermal cycler at 94, 59, and 72 C for 30 sec, 1 min, and 1 min, respectively, for 39 cycles using 50 pmol of each ERβ primer. Oligonucleotide primers were chosen from homologous parts of the coding region of the human ERβ gene. The sense primer for human ERβ was 5′-TAGTGGTCCATCGCCAGTTAT-3′, and the antisense primer was 5′-GGGAGCACACTTCACCAT-3′. Amplification of the constitutive glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was performed as an internal control for cDNA quantity and integrity, using 10 pmol of each primer of GAPDH, as described previously (25), for 28 cycles at 94, 55, and 72 C for 30 sec, 1 min, and 1 min, respectively. The PCR fragments were analyzed by 8% polyacrylamide gel electrophoresis, visualized by ethidium bromide staining, and the radioactivity was counted by Instant imager.

Results

ERβ is expressed in nuclei, similarly to nuclear ERα labeling. ERβ expression in the endometrial functional zone is usually weaker than in the positive control prostatic adenoma, and endometrial immunolabeling of ERβ is less intense than that of ERα. No immunostaining is seen in proliferative or secretory endometrium when the primary antibody is replaced by sheep IgG. Preincubation of the anti-ERβ antibody with the immunogen recombinant peptide (100 μg/mL) considerably reduces the intensity of staining (Fig. 1, A and B).

Cellular distribution of ERβ in the human endometrium

ERβ immunostaining patterns were consistent in all specimens examined. Glandular, stromal, and vascular cells all express ERβ (Figs. 1–4). ERβ expression in a particular cellular compartment varies according to the phase of the cycle (Table 1), as deduced from analysis of both staining intensity and number of immunostained nuclei with computerized image analysis (see *Materials and Methods* and Ref. 18). ERβ immunostaining intensity is low in every compartment during the menstrual phase (Table 1).

The number of labeled epithelial nuclei is higher in the proliferative phase than in the secretory phase (×1.6; $P < 0.003$), especially in the late-proliferative phase, whether the number of stained nuclei is expressed per total tissue unit area (Table 1) or per glandular unit area (data not shown); the glandular labeling intensity increases in the late-proliferative and early-secretory phases with a peak at days 14 and 15 (Fig. 1, A and D) and decreases again during the secretory phase. Stromal ERβ immunostaining is low during the menstrual cycle, except for the late-secretory phase (Fig. 1F and Table 1).

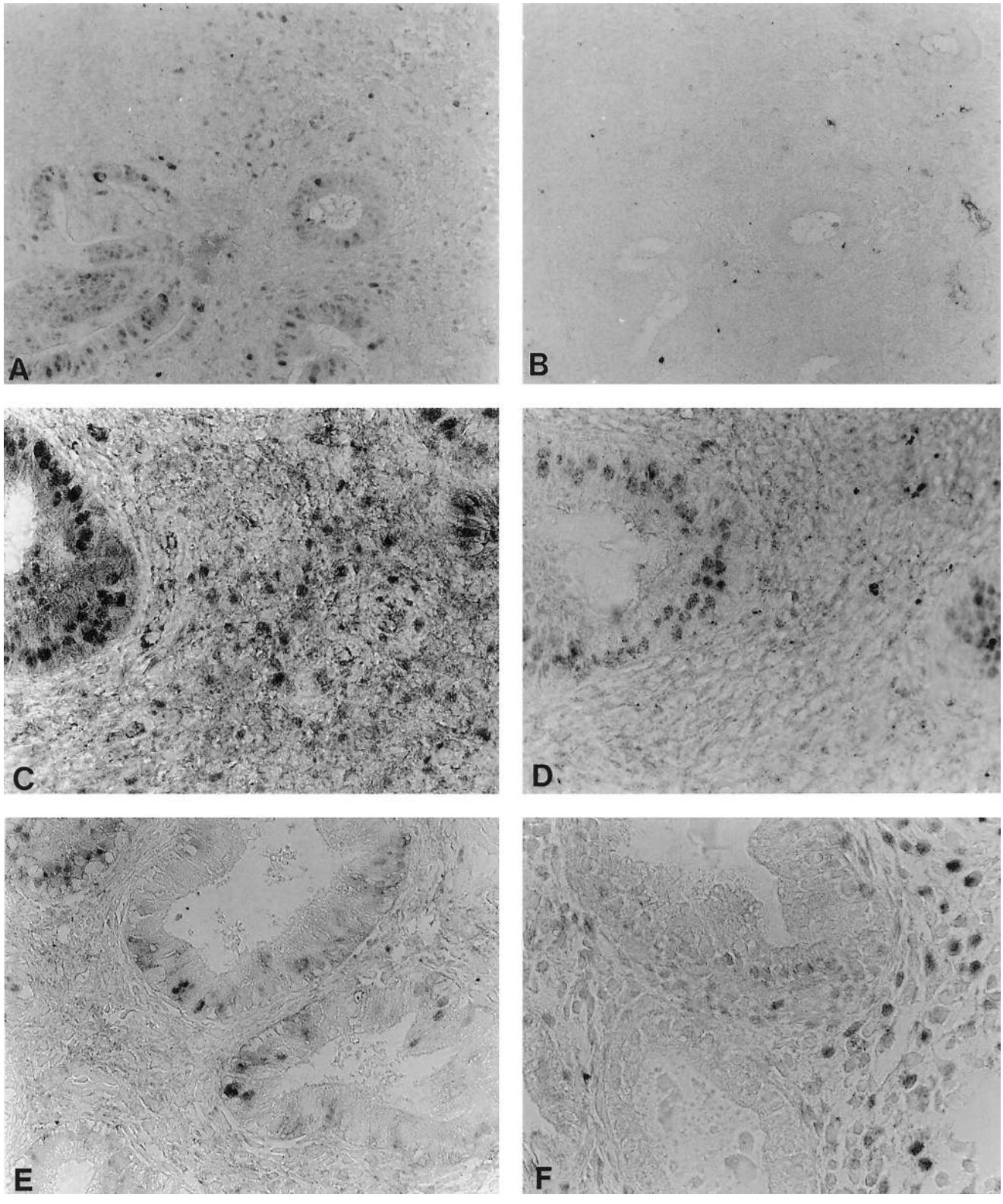

FIG. 1. Specificity of the ERβ immunostaining and expression of ERα and ERβ in endometrial structures during the menstrual cycle. Consecutive endometrial sections were incubated with anti-ERβ antibody alone (A) and preabsorbed with recombinant ERβ peptide (B, 100 μg/mL), as described in *Materials and Methods*. Note in B the disappearance of the immunostaining in glands and interstitium shown in A, when the anti-ERβ antibody has been preincubated overnight with the specific peptide. C and D, proliferative phase (day 14). E and F, secretory phase (day 25). C, strong ERα immunolabeling in glandular and interstitial cells. D, weak ERβ immunolabeling in the same structures. E, decreased glandular and interstitial expression of ERα in the secretory phase (compare with C). F, interstitial ERβ labeling has increased with respect to the proliferative period (compare with D). Original magnification, ×200.

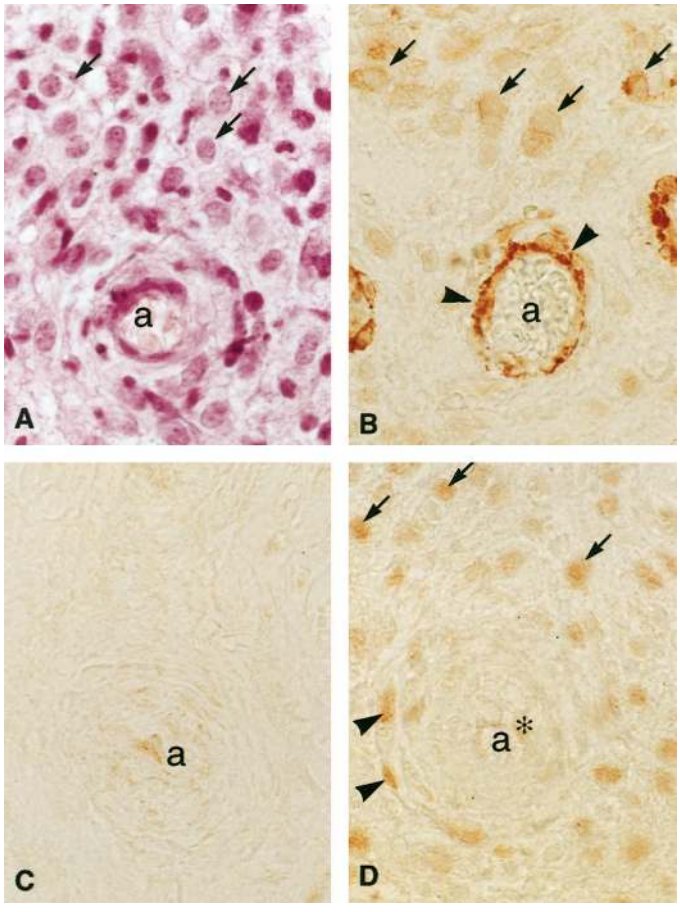

FIG. 2. Expression of ERβ in perivascular predecidual cells of the functionalis during the late secretory phase. Endometrial serial sections (day 25) were incubated with antismooth α-actin (B), anti-ERα (C), or anti-ERβ (D) antibodies, as described in *Materials and Methods*. A, hematoxylin and eosin stain showing a spiral artery surrounded by several decidual cells (*arrows*). B, smooth muscle actin positive immunostaining identifies stromal decidual (*arrows*) and vascular smooth muscle (*arrowheads*) cells. C, interstitial and vascular labeling with ERα antibody is weak at this phase of the cycle. D, anti-ERβ antibody positively stains several stromal decidual cells (*arrows*) arranged around a spiral arteriole: an endothelial cell (*asterisk*) and some smooth muscle cells of the vascular wall (*arrowheads*) are positive; a, Arteriole. Original magnification, ×600.

Vascular ERβ immunolabeling is low throughout most of the menstrual cycle; however, it increases during the late-secretory phase both in intensity (Figs. 2D and 4, B and D) and number of stained nuclei (Table 1) (×3.3 and ×2.5; $P < 0.05$, as compared with the mid- and late-proliferative periods, respectively). Vascular ERβ is predominantly expressed in parietal smooth muscle cells (CML) and occasionally in endothelial cells (Fig. 4, B and D). Thus, while epithelial ERβ immunolabeling is higher on days 14 and 15, interstitial and vascular ERβ staining peaks in the late-secretory phase.

Simultaneous or differential expression of ERα and ERβ during the cycle

It has been suggested that cell-specific effects of estrogenic action could vary according to the respective expression of ERα and ERβ and to the potential for the formation of homodimers of each ER type, as well as of ERα/ERβ heterodimers (27). To understand the cell-specific effects of estrogens in the human endometrium, we have analyzed the endometrial expression of ERα and ERβ in serial sections throughout the menstrual cycle. Our main findings concern the stronger immunolabeling of ERα compared with that of ERβ and the variations of expression of both receptors observed in the glandular, stromal, and vascular compartments according to the phase of the cycle (Table 1).

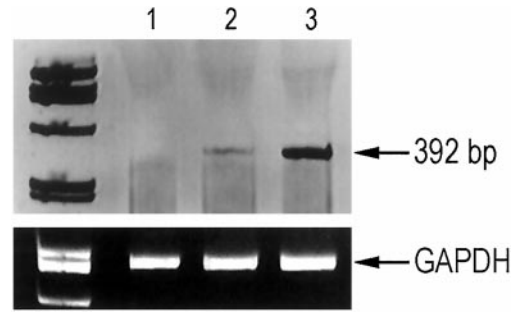

FIG. 3. Expression of ERβ transcripts in decidual stromal cells. Stromal cells were treated with EGF (20 ng/mL) plus E_2 (10^{-8} M) and progesterone (10^{-6} M) (23) for various lengths of time (1–14 days); no stimulation (lane 1), EGF + E_2 + progesterone for 6 (lane 2) or 10 days (lane 3). Total RNA was extracted from the cells at the end of each incubation, 1 μg was reverse transcribed, and an aliquot of the RT solution was amplified for 39 cycles using specific oligonucleotides for ERβ. An ethidium bromide gel of ERβ RT-PCR products from stromal cells is shown; the control (GAPDH, *bottom*) is used to standardize RT-PCR results (see *Materials and Methods* and Ref. 25). In lane 3, the product corresponding in size to the products expected for ERβ is clearly detected in cells treated for 10 days and having the characteristics of decidualized cells expressing PRL and IGFBP (23).

Proliferative phase. Glandular epithelial, stromal, and vascular cells of the functional zone express both ERα and ERβ (Table 1). ERβ immunolabeling is, however, less intense than that of ERα, especially in the glands (Fig. 1, A, C, and D). ERβ labeling in epithelial cells peaks in the periovulatory period (days 14 and 15), and glandular ERα staining is stronger during the midproliferative and periovulatory period, leading to their significant coexpression in the periovulatory period (Fig. 1, C and D).

Secretory phase. During the progression from early to late-secretory phase, glandular expression of ERα in the endometrial functional zone becomes markedly lower than in the proliferative phase, as described previously (1, 2) and confirmed in this study (Table 1). A similar pattern of immunolabeling is observed for glandular ERβ, leading to a low coexpression of ERα and ERβ in the mid- and late-secretory phase (Fig. 1, E and F). In contrast, ERβ immunolabeling in some stromal cells increases during the late-secretory period (days 25–27; Fig. 1F).

ERβ is present in stromal decidual cells of the functional zone

Stromal cells positive for ERβ during the late-secretory period (days 25–27) have a particular morphology and are mostly situated around spiral arterioles (Fig. 2); they have a round shape and the general aspect of decidual stromal cells (Fig. 2, A and D). To better characterize these cells, adjacent sections were incubated with a monoclonal antismooth α-

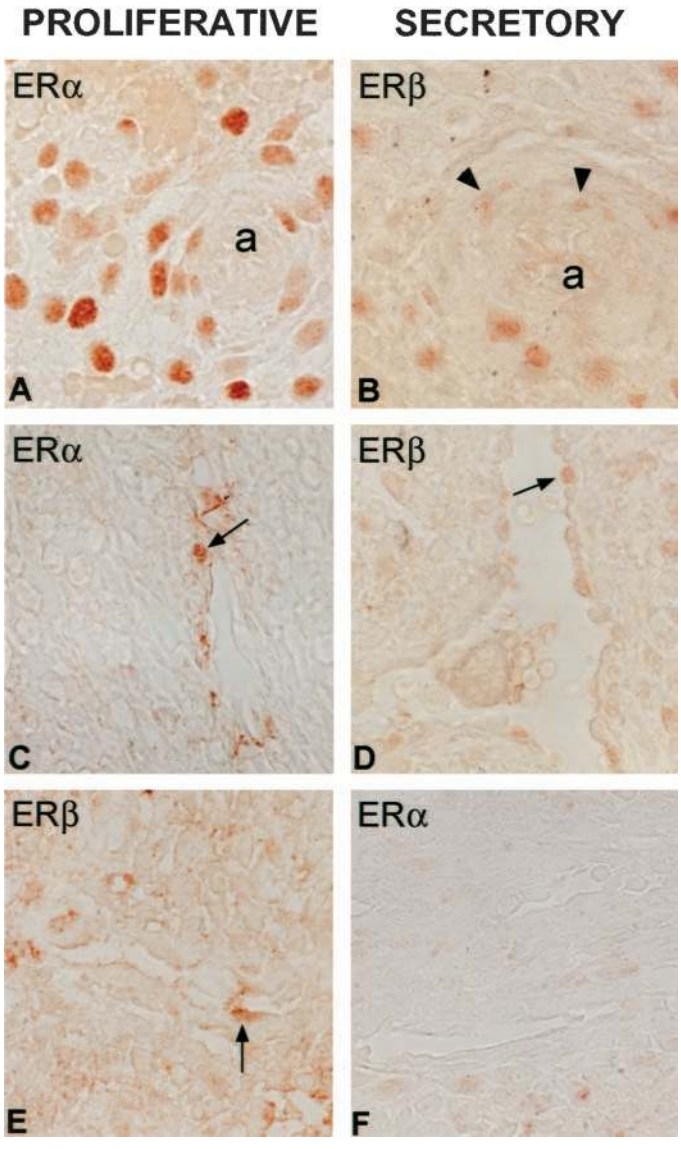

FIG. 4. Differential expression of ERα and ERβ in endometrial vessels during the menstrual cycle. A, C, and E, proliferative phase (day 14). B, D, F, secretory phase (day 25). A and C, strong expression of ERα in vascular and interstitial cells. B and D, strong immunolabeling of ERβ in arteriolar smooth muscle cells and vascular endothelium (*arrows*) at day 25. E and F, weak to absent immunostaining, respectively, for ERβ at day 14 and ERα at day 25 in interstitial cells and capillaries. a, Arteriole. Original magnification, ×400.

actin antibody, as decidual stromal cells have been shown to express this microfilament (19). As shown in Fig. 2, B and D, ERβ-positive cells are also α-actin positive, but not ERα positive (Fig. 2C).

RT-PCR analysis of human endometrial cells was used to confirm the expression of ERβ in decidual stromal cells. As shown in Fig. 3, it allows the detection of specific products having the expected size of 392 bp (ERβ). To compare ERβ transcripts in different hormonal conditions, we developed a semiquantitative RT-PCR assay (see *Materials and Methods*; Refs. 25 and 26). Stromal cells incubated with E_2, progesterone, and EGF for 10 days produce more ERβ mRNA in contrast with control cells or cells incubated for a shorter period (Fig. 3); these cells also express IGFBP, a marker of decidualization (data not shown).

Modulation of ERα and ERβ in cells of the vascular wall from the functionalis

In addition to glandular and stromal cells labeling described previously (see Refs. 1 and 2 for ERα) and reported in this study (for ERβ), nuclear ERα and ERβ immunolabeling is also present in vascular smooth muscle cells and occasionally in endothelial cells of the vascular wall (Fig. 4); a semiquantitative analysis of the immunostaining demonstrates that the expression of receptors changes during the menstrual cycle (Table 1). ERα staining of CML is restricted to the late-proliferative and early-secretory phases, when it is markedly high both in staining intensity and in number of labeled nuclei (Fig. 4A). ERβ is present in the wall of arterioles at a lower concentration than that of ERα during these periods, as deduced from the lower staining intensity (Fig. 4E). In contrast, ERβ markedly increases in vascular cells (mainly smooth muscle cells of spiral arteries) in the late-secretory period (day 25; Fig. 4, B and D), both in terms of staining intensity and number of labeled cells, whereas ERα is absent during this period (Fig. 4F and Table 1).

Discussion

Our results demonstrate the presence of ERβ in the epithelial, vascular, and stromal compartments of the human endometrial functional zone, sites known to express ERα; ERβ expression, however, is usually less prominent, and its cyclic changes are less evident than those of ERα, especially in epithelial cells (1, 2). Moreover, ERβ is the predominant form of vascular ER in the late-secretory period (days 24–26), whereas vascular ERα is mainly observed during the late follicular phase, suggesting different regulations and functions of these two ER subtypes in the vessels.

Available data on the expression of ERβ protein are restricted to animal species (8, 15, 16, 28, 29), and studies in the human endometrium have only reported the expression of the corresponding mRNAs (13, 14). Using previously characterized (15, 16) polyclonal antibodies that cross-react with human ERβ antigen, we demonstrated the immunolocalization of ERβ protein in the human endometrium; the specificity of the immunostaining was confirmed by its decrease after preincubation of the anti-ERβ antibody with the recombinant immunogen peptide, but not with the recombinant ERα protein (30); the presence of ERβ was also deduced from RT-PCR analysis in isolated endometrial and vascular cells (25). The relative levels of ERβ transcripts in uterine cells, as deduced from semiquantitative RT-PCR analysis, correlated with differences at the protein level. This simple method, which is based on the exponential nature of the PCR reaction and the use of a stable endogenous internal standard, has previously been validated using mathematical analysis and Northern blot experiments (26); however, it only permits an estimation of relative rather than absolute amounts of nucleic acids, and the results should be confirmed by quantitative methods. Compared with the presence of ERβ in human and rat uteri (13, 15), the reported absence of uterine ERβ in the mouse (8) could be related to

TABLE 1. Expression and distribution of ERα and ERβ in the human endometrium (functionalis zone) throughout the menstrual cycle

Phase of menstrual cycle	ERα			ERβ		
	Glandular staining	Interstitial staining	Vascular staining	Glandular staining	Interstitial staining	Vascular staining
Proliferative						
Days 5–9 (n = 6)	++ (485 ± 340)	+ (170 ± 49)	−/+ (5 ± 2)	−/+ (476 ± 390)	−/+ (267 ± 90)	−/+ (73 ± 43)
Days 10–14 (n = 7)	+++ (527 ± 234)	+ (287 ± 50)	++ (96 ± 20)	+ (807 ± 504)	−/+ (582 ± 104)	−/+ (100 ± 19)
Secretory						
Days 15–19 (n = 7)	++ (666 ± 208)	+ (200 ± 79)	+ (48 ± 18)	+ (388 ± 188)	−/+ (662 ± 126)	−/+ (189 ± 50)
Days 20–24 (n = 5)	−/+ (506 ± 322)	−/+ (76 ± 26)	− (4 ± 2)	−/+ (313 ± 114)	−/+ (624 ± 101)	−/+ (161 ± 26)
Days 25–28 (n = 6)	−/+ (463 ± 149)	−/+ (102 ± 30)	−/+ (20 ± 11)	−/+ (419 ± 230)	+ (703 ± 132)[a]	+ (255 ± 60)
Menstrual (n = 4)						
Days 1–4	−/+ (201 ± 127)	−/+ (134 ± 58)	− (2 ± 1)	−/+ (516 ± 394)	−/+ (134 ± 58)	− (31 ± 31)

The immunostaining was estimated semiquantitatively as: −, negative; −/+, faint; +, moderate; ++ strong; and +++, very intense, as described previously (17, 19, 21). Nuclei immunostained for ERα and ERβ were counted using computerized image analysis and the Histolab (Microvision) software program; results were expressed as number (nb) of stained nuclei × 10^3 per unit area (0.33 mm^2) ± SEM (see *Materials and Methods*).

[a] Expression decreases at days 27–28.

species differences, or to the phase of the cycle of the animals used in these experiments. The presence of ERβ in glandular epithelial and stromal cells also agrees with a previous study of the human uterus using *in situ* hybridization (13). Our results demonstrate that ERβ mRNAs are, indeed, translated into proteins in these cell types. Comparison of both the staining intensity and the number of immunolabeled nuclei for each ER within each compartment using computerized image analysis allows the analysis of relative ERα and ERβ expression in each cell type during the menstrual cycle; this methodology has been previously used for the analysis of expression of vascular endothelial growth factor and its receptors in the human endometrium (21).

Our results indicate that glandular ERβ expression predominantly occurs during the follicular phase and the early secretory period and is usually lower than that of ERα, although potential differences in the affinity characteristics of the two antibodies cannot be excluded. These findings extend and complete previous data based on mRNAs studies (13). The higher epithelial expression of ERβ and ERα on days 14–18 could modulate estrogen action in these cells at this period. The finding of the presence of ERβ in the human endometrial glands is not unexpected, although the expression of this receptor in the uterus has been reported to be low or absent (8). Disruption of the ERα gene results in some studies (11) in insensitivity to the effects of E_2 on oedema, hyperemia, RNA and DNA synthesis, mitosis, and cellular hyperplasia, whereas recent studies have shown the preservation of selective estrogen actions in the αERKO uterus (12, 31), including water retention and induction of secretory products (such as lactoferrin; Ref. 12). The maintenance of these actions by the catecholestrogen 4-hydroxyestradiol, but not by E_2, and the lack of inhibition of those effects by the pure estrogen antagonist ICI 182780 suggest the possibility of a non-ERα-mediated signaling pathway (12), possibly through activation of ERβ. Estrogenic effects, such as epithelial growth and stimulation of secretory products, have also been previously described in wild-type uterine epithelial cells lacking ERα (10, 32). Thus, preservation of selective estrogenic effects in the αERKO mouse uterus could also result from estrogenic action in ERβ-positive epithelial cells.

An important finding of our study is the presence of both ERα and ERβ in human uterine vessels, mainly in smooth muscle cells of the vascular wall and occasionally in endothelial cells, and the fact that these receptors undergo different cyclic variations. The vascular ERα increase in the late-proliferative and early-secretory phases, which has not been described before, can be explained as an effect of the estrogen preovulatory surge, similarly to the E_2-induced ERα increase observed in the rabbit uterine vessels (20). Abundant evidence in the literature shows that steroid hormones play an active role in regulating cyclic endometrial vascular changes, vascular permeability, and blood flow (see Refs. 20, 24, and 33) that are essential for endometrial physiology and for successful nidation and reproduction. Endometrial vessels regenerate at the beginning of the ovarian cycle, proliferate and branch during the follicular phase, then coil into thick-walled spiral arteries in the luteal period to undergo collapse and sloughing at menses (24). The observation of the presence of ERs at relatively high concentrations in smooth muscle cells of the vascular wall of uterine vessels (18, 20) has led to the conclusion of a direct action of this steroid on endometrial vasculature. Activation of vascular ERα receptors during the periovulatory period could be related to vasodilation, the production of paracrine factors in the vascular smooth muscle cells and their permeabilizing action on the vessels (20, 22, 34).

In the mid-late secretory phase (days 24–26), the finding that ERβ is the predominant form of vascular ER, while vascular ERα levels are very low, suggests that ERβ might play a role in the regulation of vascular function in this specific phase of the cycle. Vascular ERβ is expressed both when the coiling of the spiral arterioles reaches its maximal level and when the decidual changes of the stromal cells surrounding those same arterioles occur (24); also ERβ increases in late-proliferative decidual stromal cells and in decidual cells during pregnancy (unpublished data), suggesting some specific role of this receptor during the process of decidualization. Mice lacking ERβ have been reported to be fertile, but have fewer and smaller litters than wild-type mice (35); this result was interpreted as the consequence of a direct loss of ERβ-mediated actions in the ovary. However, the ability of progesterone to in-

duce a decidual response in the absence of ERα in endometrial stromal cells of αERKO mice with high E_2 levels (36) is a finding that could be explained through activation of the ERβ in addition to activation of the progesterone receptor. The reasons for the appearance of substantial levels of ERβ in human vascular and stromal cells during the mid-late luteal transition (days 24–26) and the disappearance of the receptor during hormonal withdrawal (days 27–28) remain to be elucidated.

Differences in tissue distribution of ERβ and ERα during the menstrual cycle suggest the presence of analogous or distinct roles fulfilled by each receptor. ERβ may, therefore, play a role in the modulation of estrogenic action, either in combination with ERα or by itself. It has been suggested that the potential for the formation of homodimers of each ER type, as well as heterodimers, can have differential effects on gene activation (27). Moreover, ERβ has been demonstrated to exert opposite transcriptional effects in comparison to ERα, after binding to estrogens and antiestrogens in some promoter context (37). The transcriptional activity in estrogen-responsive tissues may, therefore, be modulated by the ER-type and the relative content of each receptor. ERβ may modulate the activity of ERα in glandular cells and possibly in cells of the vascular wall, especially during the late-proliferative and early-secretory phases when these cells co-express the two receptors. Differences in the ERα/ERβ ratio among different hormonal conditions and in pathological disorders (38–39) could have important functional implications, because these ERs have different binding characteristics (7) and different levels of expression are found in pathological conditions such as endometriosis (38, 39). We also postulate that a decrease in the ERα/ERβ ratio in stromal and vascular cells at day 25 could influence endogenous gene expression during the decidualization process.

In summary, this study indicates the presence of ERβ receptors in epithelial, stromal, and vascular cells of the human endometrium and describes their cyclic changes during the menstrual cycle. Our findings may explain the preservation of selective actions of estrogens in the α-ERKO mouse uterus, and provide a basis for the reinvestigation of specific actions of E_2 that are not mediated by ERα in the human endometrium. Further investigations into the ERβ-mediated signaling pathway, by which physiological estrogens or xeno-estrogens may activate nuclear processes, are needed to better elucidate the role of this receptor in endometrial cyclic functional changes.

Acknowledgments

We thank Dr. P. Saunders (Edinburgh, England) for the gift of anti-ERβ antibodies and Profs. G. Caruso and L. Resta (Bari, Italy) for the gift of human endometrial biopsies. We thank Prof. G. Feldmann (INSERM U 327, Paris, France) and P. Loiseau (CHU Xavier Bichat, Paris, France) for help in computerized image analysis.

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