Estrogen Receptor α and Progesterone Receptor in the Rhesus Endometrium During the Late Secretory Phase and Menses (44298)

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Abstract. **During menses in the primate, zone I and parts of zone II of the endometrium are sloughed, and subsequently reconstruction of the endometrium for the next cycle begins. In this study we examined the cell type and zonal changes in estrogen (E) and progesterone (P) receptors (R) that occur during these changes in endometrial structure and the coincident changes in hormonal stimulation. Immunohistochemical analyses of ERα and PR were performed on endometrial biopsies obtained during artificial menstrual cycles in the rhesus monkey on day 26 (declining serum P), Days 1 and 3 of menses, and Day 5 (following endometrial reconstruction). The pattern and distribution of ERα and PR in the endometrium on Day 26 was similar to that observed previously on Day 23; ERα and PR showed strong positive staining for glandular epithelia in zone IV of the basalis and little or no staining for ERα in zones I and II-III whereas PR showed positive staining in stromal cells and little or no staining in epithelia in zones I and II-III. On Day 1 of menses, no detectable ERα and PR staining was observed in glandular epithelial throughout the endometrium. PR immunoreactivity remained in stromal cells on Day 1 whereas ERα staining returned to stromal cells reflecting a release from P inhibition of ERα. By Day 3 of menses positive staining for ERα and PR was observed in glandular epithelia in zones II-III and zone IV. By Day 5 the reconstruction of the endometrium was complete, and strong positive staining for both receptors was present throughout the endometrium. Following removal of all E stimulation on Day 26, no distinct differences in receptor distribution were observed on Day 3. These data suggest that hormone-independent mechanisms such as in normal wound healing are also operative during menses and reconstruction. The presence of ERα, primarily in stromal cells during normal menses, supports the notion that the mesenchymal compartment plays an important role in the orchestration of normal endometrial reconstruction in response to E.** [P.S.E.B.M. 1998, Vol. 218]

In the absence of implantation, the decline in serum P during the late secretory phase in the primate menstrual cycle triggers the process of menstruation. The loss of endometrial vascular integrity that occurs during menses leads to bleeding and shedding of tissue (1, 2). During endometrial shedding there is little proliferative activity in the remaining endometrium until bleeding ceases (3). These events occur during a striking change in hormonal balance: a progesterone (P) dominated endometrium (late secretory phase) becomes an estrogen (E) dominated endometrium (early proliferative phase).

Because of their central role in endometrial growth and differentiation, this study focused on the regulation of ERα and PR in the rhesus endometrium during menses and regeneration by immunohistochemical analyses. Menses serves as a dramatic sign of the transition from a P-dominant (late secretory phase) to an E-dominant (early proliferative phase) endometrium. During natural menstrual cycles, it is often difficult to obtain precisely timed endometrial samples for analysis because of inherent animal to

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animal variation. We circumvented this potential problem by the use of ovariectomized rhesus monkeys in which the hormonal pattern of the natural menstrual cycle was simulated by sequential insertion or removal of silastic implants packed with crystalline E and P (4). Exogenous control of titers of serum E and P during these artificial menstrual cycles allowed us to obtain endometrial tissue at more precise time points.

Previous studies from our laboratory (5–7) and others (8–11) have demonstrated the zonal and cell-type changes in ER and PR immunostaining during the changeover from an E-dominant (late proliferative phase) to a P-dominant (midsecretory phase) endometrium in the nonhuman primate and human. The present study extends our previous findings on endometrial proliferation during menses and provides a basis for interpretation of E-dependent changes in proliferation during this hormonal transition.

Materials and Methods

Animals. Mature female rhesus monkeys (*Macaca mulatta*) obtained from commercial sources (Hazelton Labs, Alice, TX) were ovariectomized for at least 2 months prior to initiation of artificial menstrual cycles. All protocols were approved by the Institutional Animal Care and Use Committee. Artificial menstrual cycles and E and P were measured by RIA as previously described (4, 6). Serum E and P in these artificial menstrual cycles have been shown (3, 4, 6, 12) to mimic those observed during the natural cycle (13).

Immunohistochemical Analyses of ERα and PR. Endometrial biopsy tissue was obtained from the fundal region of the uterus by hysterotomy as described previously (14). Previous work by Tsibris *et al.* (15) has shown regional differences in the concentration of E and P receptors in the human endometrium. Our biopsy samples were restricted to the fundal region of the uterus to minimize variation.

The ER antibody used in these studies recognizes the α and not the β form of the receptor (GR17, Oncogene Sciences, Cambridge, MA). The PR antibody recognizes both A and B isoforms (GR18, Oncogene Sciences). The avidin-biotin complex technique was used for immunohistochemical localization (Vector Labs, Burlingame, CA). Immunohistochemical analysis of ERα and PR in these studies was as previously described by our laboratory (5, 6). All incubations with immunochemicals were performed in a humidified chamber. Briefly, sections were treated with ERα antibody or control (normal rat IgG) at 2.5 μg/ml in PBS overnight (16–18 hr) at 4°C. For PR localization, sections were treated with PR antibody or preimmune mouse IgG (5 μg/ml) overnight at 4°C. Sections were washed and incubated with biotinylated secondary antimouse IgG (Vector Labs). Incubations were conducted with secondary antibodies for 1 hr at room temperature. Sections were then washed and incubated (30 min, RT) with streptavidin-peroxidase complexes. The antigen-antibody complex was detected by incubation with 3′5′-diaminobenzidine (DAB) solution (0.5 mg/ml) in Tris-HCl (0.05 *M*, pH 7.6) containing 0.0025% hydrogen peroxide. Slides were dehydrated through ascending grades of ethanol, cleared in xylene, and mounted with Permount (Fisher, Fair Lawn, NJ). Photomicrographs were made with a Zeiss photomicroscope (Carl Zeiss Inc., New York, NY) using Ektachrome 400 (Kodak, Rochester, NY) and Zeiss pan-neofluar lenses at magnifications of 200× or 400×. Methodological controls included omission of the first antibody or second antibody or streptavidin-peroxidase complex or omission of all of the above with the DAB detection system alone. No specific staining was observed with the above controls.

Results

As the serum P level began to fall on Day 26 in the late secretory phase (3), the pattern of ERα distribution in the endometrial zones remained similar to that observed previously on Day 23 (5). That is, little or no ERα immunostaining was found in the stroma or luminal and glandular epithelia of zones I and II-III whereas strong positive staining remained in glandular epithelia in zone IV of the basalis (Fig. 1a, far right). With the use of these immunohistochemical techniques, all staining was localized to the nucleus for both ERα and PR. On Day 1 of menses, when serum P level was below the level of detection (3), no detectable ERα staining of glandular epithelia was observed throughout the endometrium including zone IV (Fig. 1b). Positive staining for stromal ERα, however, was found in all zones of the endometrium at this time. Although all endometria showed evidence of sloughing on Day 1 of menses, a sample that contained a portion of the luminal epithelium (zone I) on this day was shown in Figure 1b for comparison to Day 26.

As the process of repair and reconstruction of the endometrium continued on Day 3, there was little or no ERα staining in epithelia (luminal and glandular) throughout the endometrium (Fig. 2a). Strong stromal cell staining for ERα was present in all zones of the endometrium. By Day 5, strong positive staining for ERα returned to both luminal and glandular epithelia in all zones of the endometrium (Fig. 2b). The pattern and distribution of ERα immunoreactivity on Day 5 was similar to that observed previously on Day 13 (6) at the height of E stimulation.

The regulation of PR during this transition from a P-dominant to an E-dominant endometrium was similar to that of ERα with a few notable differences. On Day 26 the endometrium showed positive PR staining in stromal cells throughout the endometrium with no detectable staining in glandular epithelia of zones I and II-III (Fig. 3a). This result contrasts with ERα staining on Day 26 wherein staining is absent from stromal cells and weakly expressed in glandular epithelia. Similar to the pattern of ERα staining, glandular epithelia in zone IV show strong positive staining for PR on Day 26. As noted previously, this pattern of ERα and PR localization is conincident with zone IV epithelial proliferation on both Day 23 and Day 26 (5–7).

PR staining is absent from epithelia in all zones of the

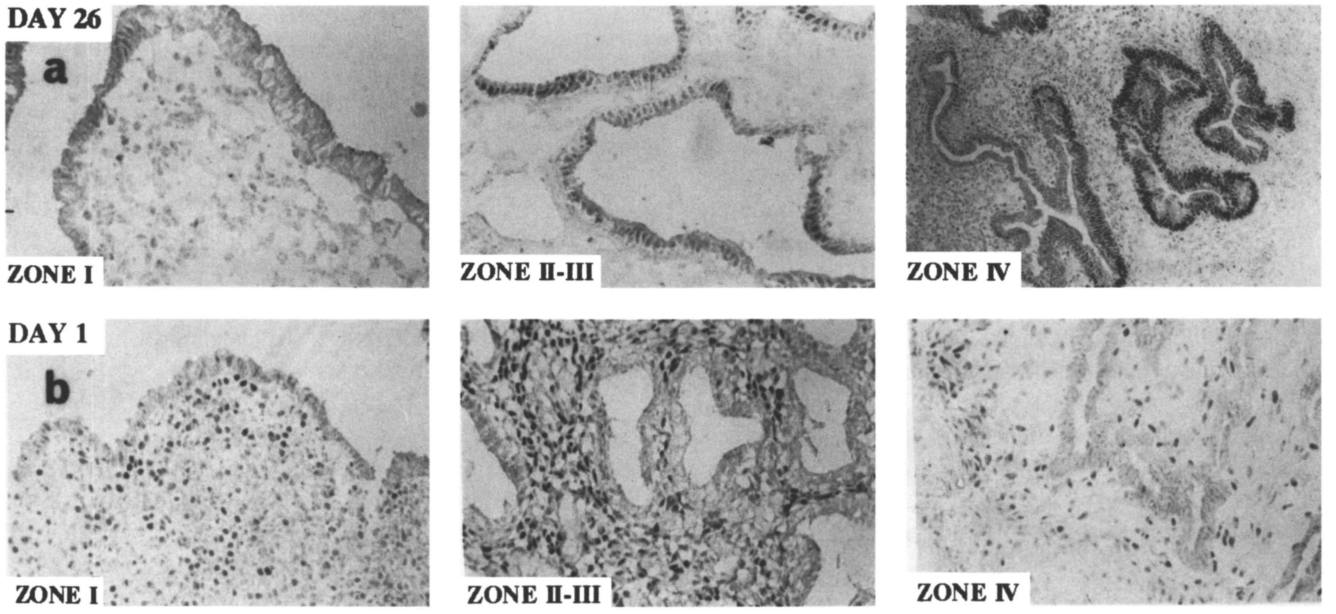

Figure 1. ER distribution in the rhesus monkey endometrium on Days 26 and 1 in the artificial menstrual cycle. (a) Day 26, (b) Day 1. Endometrial zones are indicated in each panel. Data shown are representative of three to four animals. Magnification: 200×.

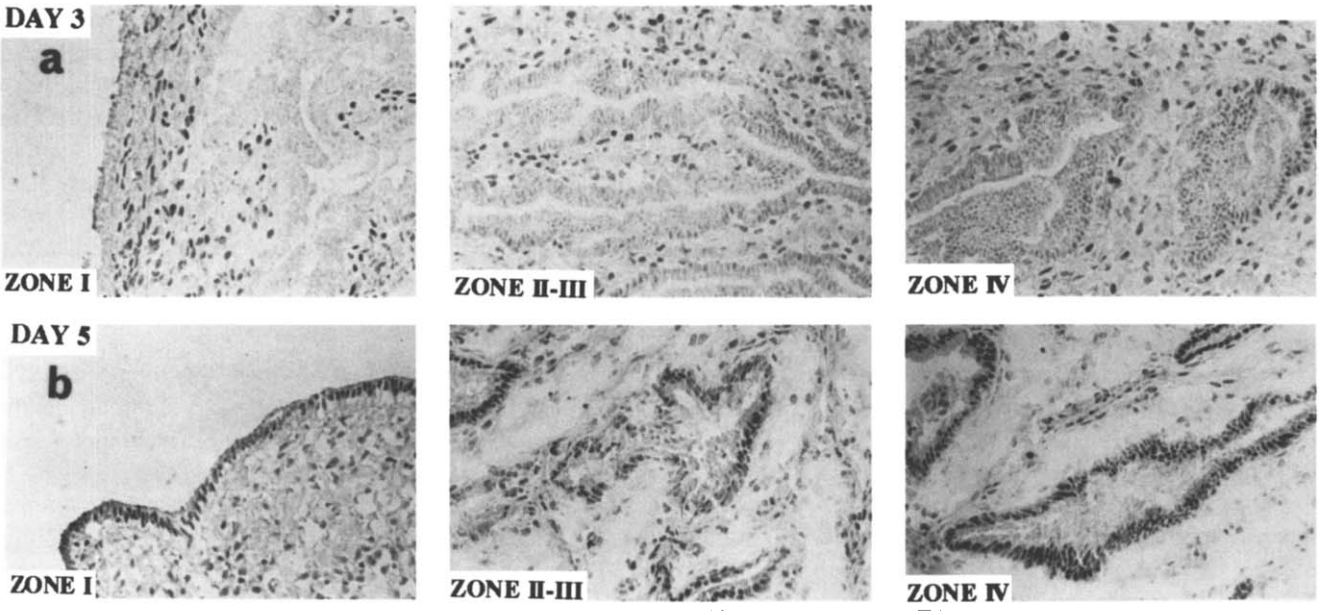

Figure 2. ER distribution in the rhesus monkey endometrium on Days 3 and 5 in the artificial menstrual cycle. (a) Day 3, (b) Day 5. Endometrial zones are indicated in each panel. Data shown are representative of three to four animals. Magnification: 200×.

endometrium on Day 1 of menses whereas stromal fibroblasts throughout the endometrium retain PR immunoreactivity (Fig. 3b). On Day 3, only scattered weak staining is observed in glandular epithelia (Fig. 4a). Following the reconstruction of the endometrium on Day 5, strong positive staining for PR is present in luminal and glandular epithelia and stromal fibroblasts throughout the endometrium (Fig. 4b). This pattern of PR staining on Day 5 is similar to that observed previously on Day 9 of artificial menstrual cycles (5).

The use of timed placement and removal of E and P implants to simulate the natural cycle also allowed us to alter the steroid milieu to study specific hormonal responses. We removed all E stimulation (basal E, 50–70 pg/ml) on Day 26 and analyzed the pattern of ER and PR staining on Day 3 in zones II-III. Although strong positive staining for both ERα and PR observed in the stromal compartment, ERα and PR was staining in glandular epithelia is weak (Fig. 5a). These results are similar to those seen in the presence of basal E.

Discussion

The use of immunohistochemical techniques to localize ERα and PR within the primate endometrium is an impor-

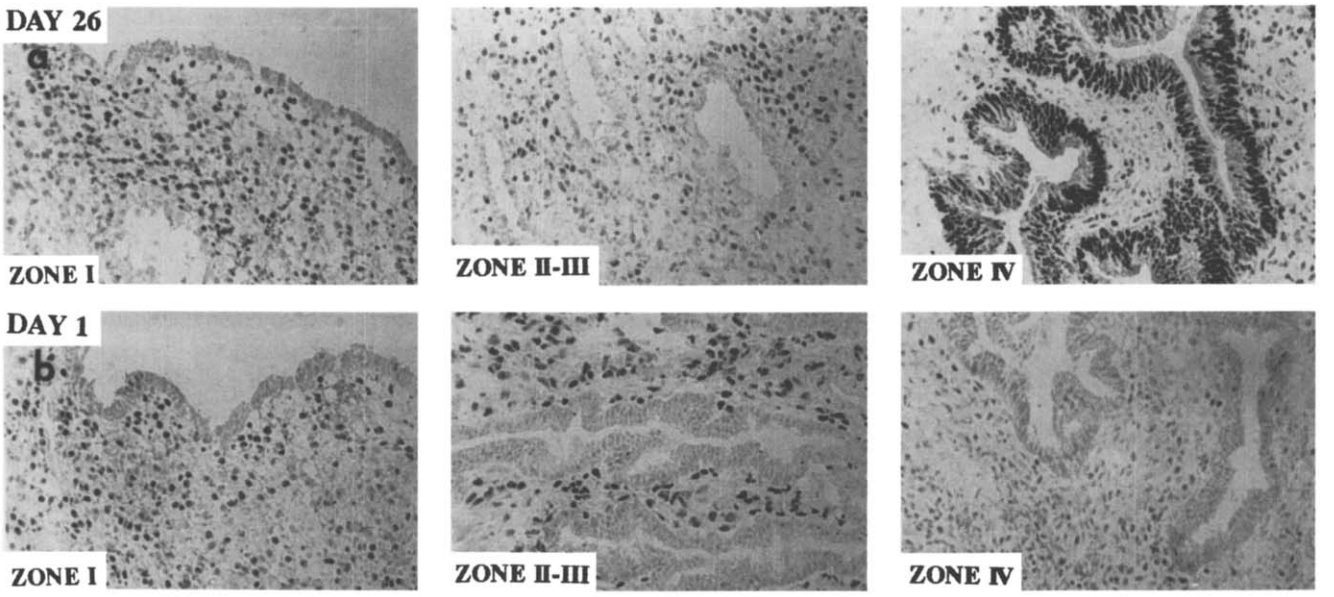

Figure 3. PR distribution in the rhesus monkey endometrium on Days 26 and 1 in the artificial menstrual cycle. (a) Day 3, (b) Day 5. Endometrial zones are indicated in each panel. Data shown are representative of three to four animals. Magnification: 200×.

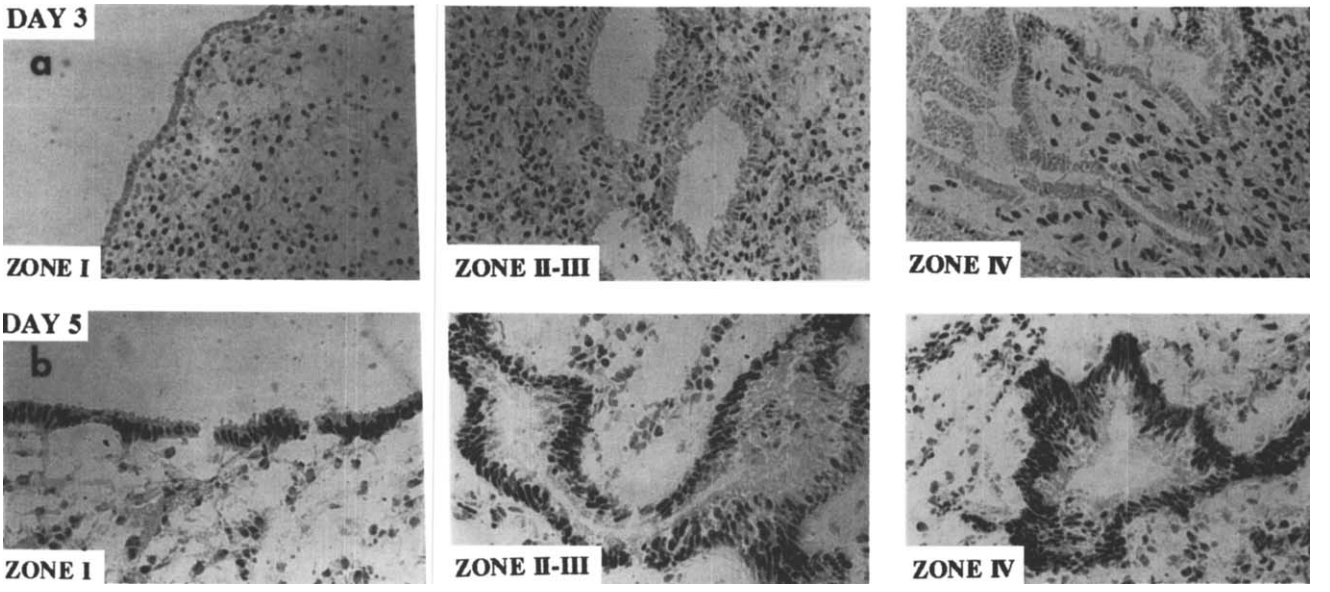

Figure 4. PR distribution in the rhesus monkey endometrium on Days 3 and 5 in the artificial menstrual cycle. (a) Day 3, (b) Day 5. Endometrial zones are indicated in each panel. Data shown are representative of three to four animals. Magnification: (a) 200×, (b) 400×.

tant approach that extends previous traditional biochemical analyses. In the primate endometrium, this approach is of particular value because of the classical studies of Bartelmez (16–17) that demonstrated a horizontal bipartite zonation based on epithelial, stromal and vascular differences. In addition, the uterus is composed of several different cell types that have been shown to respond differently to the same hormonal stimulation (18–19). Different cell-type responses to the same hormonal stimuli suggest an important role in the coordination of hormonal signals that permit the uterus to achieve appropriate endpoints in reproduction.

The endometrial regression and reconstruction that occur during the menstrual cycle are accompanied by dramatic changes in the hormonal milieu. The loss of P influence in the late secretory phase leads to regression and sloughing of the endometrium and subsequently, E becomes the dominant hormonal influence during reconstruction. Our present study examines all zones of the rhesus endometrium in artificial menstrual cycles that mimic the rising and falling levels of E and P observed in the natural menstrual cycle. Although our protocol does not replace ovarian androgen secretion (20), a similar protocol in ovariectomized rhesus monkeys has been shown to provide adequate endometrial maturation for surrogate embryo transfer and subsequent delivery (21). These studies not only complete an analysis of the spatial and cell-type regulation of ER and PR during the rhesus menstrual cycle but also provide an important companion to our previous published studies on prolifera-

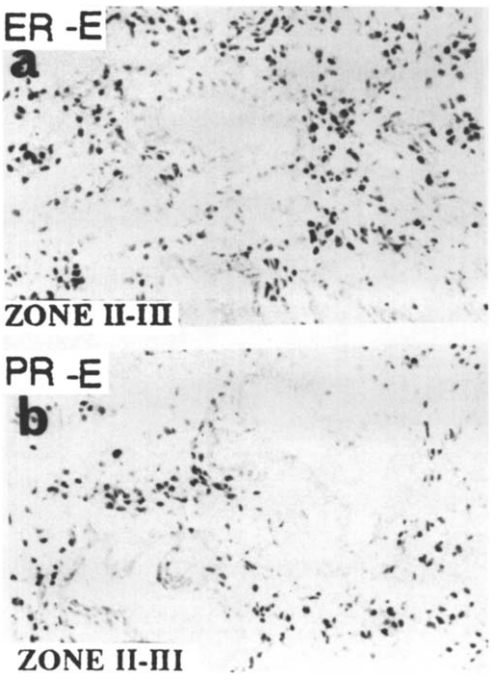

Figure 5. ER and PR immunostaining in the rhesus monkey endometrium on Day 3 in the absence of E stimulation. (a) ER, (b) PR. Data shown are representative of three to four animals. Magnification: 200×.

tion during menses and reconstruction. Our results demonstrate distinct differences in cell-type and zonal patterns of ERα and PR expression during this transition from a P-dominant endometrium to an E-dominant endometrium.

The distribution of ERα and PR on Day 26 (falling serum P) is similar to that observed previously on Days 21 and 23 (5–6) (i.e., ERα and PR are primarily localized in zone IV epithelia, and PR but not ER is present in stroma throughout the endometrium). Similar cell-type differences in ER and PR localization during the late luteal phase in the human (10, 22–25) and nonhuman primate (26–27) have been described previously.

On Day 1, glandular epithelia in the basalis zone IV lose ERα and PR immunoreactivity; all epithelia lack detectable ERα and PR. These data suggest that on Day 1 of menses, glandular epithelia are incapable of responding directly to either E or P. Consistent with the absence of ERα in glandular epithelia on this day, our previous studies have shown little or no proliferation in these cells despite the presence of basal serum E level (50–70 pg/ml) and undetectable levels of serum P (3). In contrast, stromal cells throughout the endometrium contained ERα and PR but also showed little or no proliferation suggesting that the block in E-dependent proliferation at this time during menses is independent of ERα.

On Day 3 of menses stromal ERα and PR are prominent in the stromal compartment whereas glandular and luminal epithelia show little or no ERα or PR. Surprisingly, the absence of ERα in glandular epithelia of zone II-III is coincident with a high level of proliferation of these cells at this time (3). A previous study in the rhesus monkey also reported endometrial DNA synthesis of such ER negative glandular epithelial cells following P withdrawal (28). Importantly, recent studies have provided insight into the mechanism of this apparent paradox. Although stromal directed epithelial function has been described for several tissues (29), the ERα knockout mouse (ERKO) has provided an avenue for testing the importance of uterine stromal influence on ERα-negative epithelial cells. Cooke *et al.* (30) used uterine tissue recombinants (e.g., wild type stroma (ERα-positive) and ERKO epithelia (ERα-negative)) to demonstrate that epithelial proliferation in response to E is a paracrine-mediated response *via* stromal cells. Our studies, taken together with our previously published data (3) provide further *in vivo* support for stromal-directed regulation of endometrial epithelial cell proliferation.

By Day 5 the endometrium has the appearance of a typical proliferative endometrium (i.e., ERα and PR are present in stroma and luminal and glandular epithelia in all endometrial zones). Despite the presence of ERα staining in zone IV glandular epithelia by Day 5, little proliferation is observed in this zone at this time (3). Proliferation in zone IV glandular epithelia is blunted despite the presence of ERα. Whether or not this blunted response to E in zone IV is a result of local (stromal-derived) inhibitory factors requires further study.

Recently, a new ER, designated β, has been cloned in the rat (31), mouse (32), and human (33). ERβ also shows high affinity for estradiol and has been shown to be present in the rodent uterus although at lower levels than other tissues such as the prostate and ovary (34). Despite the presence of uterine ERβ in the ERKO mouse, there is no uterotrophic response to E (35). Thus, the presence of ERα, as described in the present study, appears to be necessary for E-dependent proliferation and uterine growth. The discovery of ERβ can potentially provide an important pathway for understanding the pleiotrophic actions of estrogens and will doubtlessly be the subject of numerous studies in the future (36).

Our studies have analyzed the stromal and epithelial presence of ERα and PR in all zones of the rhesus endometrium during menses and reconstruction. These data compliment our previous report on proliferation during this major hormonal transition in the rhesus menstrual cycle (i.e., P-dominant to E-dominant endometrium). These data also further attest to the unique spatial and temporal hormonal responsiveness of the primate endometrium and provide evidence for different hormonally responsive microenvironments within the endometrium. How these different patterns of receptor regulation may govern the varied responses of the endometrium during menses and reconstruction will require further studies.

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