Paracrine Regulation of Epithelial Progesterone Receptor and Lactoferrin by Progesterone in the Mouse Uterus

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ABSTRACT

The objective of this study was to determine whether uterine stromal and/or epithelial progesterone receptor (PR) is required for the antagonism by progesterone (P4) of estradiol-17β (E2) action on expression of PR and lactoferrin in uterine epithelium. Uterine tissue recombinants were prepared with epithelium (E) and stroma (S) from wild-type (wt) and PR knockout (PRKO) mice: wt-S+E and PRKO-S+E. P4 action on epithelial PR expression was studied in wt-S+E and PRKO-S+E tissue recombinants. E2 down-regulated epithelial PR in both types of tissue recombinants, but P4 blocked E2-induced down-regulation of epithelial PR only in wt-S+E tissue recombinants. Thus, P4 requires stromal PR to inhibit E2-induced down-regulation of epithelial PR. Epithelial PR is not sufficient in itself. The inhibitory effect of P4 on lactoferrin expression was studied in 4 types of tissue recombinants (wt-S+E, PRKO-S+E, wt+S+PRKO-E, and PRKO-S+PRKO-E). E2 induced lactoferrin in all 4 types of tissue recombinants. P4 blocked E2-induced lactoferrin expression only in wt-S+E tissue recombinants. In wt-S+PRKO-E tissue recombinants, P4 inhibited lactoferrin expression only partially. P4 failed to block E2-induced lactoferrin expression in PRKO-S+wt-E and PRKO-S+PRKO-E tissue recombinants. Thus, both epithelial and stromal PR are essential for full P4 inhibition of E2-induced lactoferrin expression.

INTRODUCTION

Two major ovarian steroids, progesterone (P4) and estradiol-17β (E2), are key regulatory components of reproduction in females. Uterine function is coordinately regulated by P4 and E2, and in many cases P4 antagonizes the effect of E2. For example, in the mouse uterus, E2 is mitogenic in vivo while P4 inhibits E2-induced proliferation of endometrial epithelium [1]. P4 also inhibits expression of E2-regulated genes such as Muc-1 [2] and C3 [3] in the mouse uterine epithelium. Both P4 and E2 elicit their functions through binding to specific intranuclear proteins—progesterone receptors (PR), and estrogen receptors (ER), respectively. Mice with targeted disruption of the PR gene (so-called PR knockout [PRKO] mice) exhibit impaired reproduction due to abnormalities in behavior, as well as abnormalities in progesterone target organs. In the uterus, the absence of PR results in epithelial hyperplasia due to the unopposed mitogenic action of E2 [4].

The PR gene itself is one that is regulated by both E2 and P4. In many mammalian species, PR is expressed in most major cell types of the uterus: luminal and glandular epithelium, endometrial stroma, and myometrium. In humans [5], primates [6], sheep [7], cats [8], and pigs [9], PR is up-regulated by E2 and down-regulated by P4 in most uterine cells, including epithelial cells. However, PR is down-regulated by E2 in uterine epithelium of rats and mice [10–14]. In the accompanying paper, we have demonstrated that E2 down-regulates uterine epithelial PR through ERα in uterine stroma [15]. Thus, uterine epithelial PR is regulated by E2 via a paracrine mechanism.

Lactoferrin is one of the major estrogen-regulated secretory proteins in the murine uterine epithelium, and its expression is induced by E2 and inhibited by P4 [16]. A previous study has shown that both epithelial and stromal ERα are essential for E2-induced lactoferrin expression [17]. Thus, regulation of lactoferrin by E2 in uterine epithelium involves both direct and paracrine action of E2 through epithelial and stromal ERα, respectively.

The objective of this study was to determine whether P4 elicits its effect on expression of PR and lactoferrin in uterine epithelial cells via epithelial and/or stromal PR. Both E2-induced down-regulation of PR and E2-induced up-regulation of lactoferrin require stromal ERα. Given the importance of paracrine pathways in uterine biology, it is essential to determine whether epithelial and/or stromal PR is required for P4 antagonism of E2 actions on expression of PR and lactoferrin in the mouse uterus epithelium. Effects of P4 on uterine epithelial PR in mice and rats have not been studied adequately even though P4 has been reported to inhibit E2-induced down-regulation of uterine epithelial PR in rats [12] but not in mice [14].

In this report, we demonstrate the inhibitory effect of P4 on E2-induced down-regulation of mouse uterine epithelial PR. In ovariectomized mice, PR was highly expressed in uterine epithelium. E2 down-regulated uterine epithelial PR, and P4 partially inhibited the E2-induced down-regulation of PR protein and mRNA in murine uterine epithelium. Tissue recombination experiments utilizing PRKO mice demonstrated that uterine epithelial PR was regulated by P4 through novel paracrine mechanisms mediated via stromal PR. Uterine epithelial PR itself was not sufficient to block E2-induced down-regulation of epithelial PR. Tissue recombination experiments also showed that P4 can partially inhibit the E2-induced lactoferrin expression via stromal PR, but both epithelial and stromal PR were essential for full antagonism of P4 on E2-induced lactoferrin expression. Thus, lactoferrin is regulated by P4 through both a direct action via epithelial PR and an indirect paracrine mechanism via stromal PR.

MATERIALS AND METHODS

Animals and Treatments

All animals were maintained in accordance with the NIH Guide for Care and Use of Laboratory Animals, and all

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procedures described here were approved by the University of California-San Francisco and University of Illinois animal care and usage committees. Mice were maintained under controlled temperature and lighting conditions during the experiment, and were given food and water ad libitum. Adult female athymic nude mice and adult female CD-1 mice were purchased from Charles River (Wilmington, MA). PRKO mice were produced as described previously [4].

All hormone injections were i.p. in 0.1 ml peanut oil (Sigma, St. Louis, MO). To study the effect of P₄ on expression of PR and lactoferrin, hormone treatments were adapted from previous studies [2, 15–17] with modifications. Daily dosages of 125 ng E₂ (Sigma) and 2 mg progesterone (Steroids Inc., Wilton, NH) were used. In all experiments, hormone treatment was started 2 wk after ovariectomy. Ovariectomized female CD-1 mice (approximately 90 days old) and host athymic nude mice for PRKO/wild-type (wt) uterine tissue recombinants received hormone treatments on the following schedule: oil group, oil only on Days 1–4; E₂ group, oil on Day 1, E₂ on Days 2–4; E₂+P₄ group, P₄ on Day 1, E₂+P₄ on Days 2–4; P₄ group, P₄ on Days 1–4. Twenty-four hours after the last hormone injection, animals were killed to harvest tissue samples. In the tissue recombinant experiments, vaginae and uteri were also collected from host mice as controls.

Tissue Separation, Recombination, and Grafting

Procedures for separation and recombination of epithelium and stroma from mouse uteri have been described previously [18]. Details of uterine tissue recombination with PRKO mice have been described previously [19]. Briefly, uteri from PRKO or wt litter mates were dissected free of connective tissue and fat, placed into Hanks’ Balanced Salt Solution (HBSS), and cut into 4–6 segments/uterine horn. The pieces were enzymatically dissociated by trypsin digestion, uterine epithelium was flash-frozen in liquid N₂. Uteri from at least 2 litters were enzymatically separated using fine surgical instruments. Stroma and epithelium were physically separated using fine surgical instruments. Stroma and epithelium were recombined on nutrient agar plates and allowed to adhere during overnight culture at 37°C. After trypsin digestion, uterine segments were opened longitudinally with spring-loaded Vannas scissors, and then stroma and epithelium were physically separated using fine surgical instruments. Stroma and epithelium were recombined on nutrient agar plates and allowed to adhere during overnight culture at 37°C. After overnight culture, the tissue recombinants were grafted under the renal capsules of female athymic nude mice. Approximately 1 mo after grafting, all hosts were ovariectomized and then received the hormone treatments described above.

Immunohistochemistry

Anti-human PR rabbit polyclonal IgG and anti-human ERα mouse monoclonal IgG 1D5 were purchased from DAKO (Carpenteria, CA). Rabbit antiserum against lactoferrin was obtained from Dr. Christina Teng (National Institute of Environmental Health Science, Research Triangle Park, NC). Tissues were fixed with 4% paraformaldehyde for 3 h on ice, processed into paraffin, and then sectioned at 6 μm. Immunohistochemical detection of ERα [20] and PR [19] in mouse tissues has been described. Immunoreactivity was developed for ERα and PR by a Vectastain Elite ABC kit (Vector Laboratories Inc., Burlingame, CA). For lactoferrin immunohistochemistry, tissue sections were deparaffinized through series of xylene and ethanol, and then sections were incubated overnight with lactoferrin antiserum (1:200 in PBS) or nonimmune normal rabbit serum (Zymed Laboratories, Inc., South San Francisco, CA). Sections were then incubated with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG (Sigma), and the lactoferrin immunoreactivity was visualized by fluorescent microscopy. No signal was observed in the negative control with nonimmune rabbit serum (data not shown).

Image Analysis

Morphometric analysis of PR immunohistochemistry has been described [15]. For morphometric analysis of PR immunohistochemistry, epithelial nuclei were manually picked up by adjusting the threshold from immunostained slides, and then the optical density (OD) in the yellow channel of the CMYK (cyan, magenta, yellow, black) mode was measured in the epithelial nuclei. The average OD of controls with nonimmune IgG was used as the baseline (OD = 0). In each group, approximately 5000 nuclei were analyzed from 5 to 8 samples. For morphometric analysis of lactoferrin immunohistochemistry, fluorescent images of lactoferrin immunohistochemistry were captured with a DC330 camera (Dage-MTI Inc., Michigan City, IN) interfaced with a PowerBase 200 computer (Power Computing, Round Rock, TX) and analyzed with Scion Image 1.62a (Scion Inc., Frederick, MD). Areas of epithelia were manually selected from the brightfield images, and the OD of the FITC images was measured in the selected area. In each group, approximately 1000 cells were analyzed from 4 to 8 tissue recombinants of 3–5 independent experiments. Statistical analysis was performed using the StatView software (Abacus Concepts Inc., Berkeley, CA). To compare OD for different hormone treatments, ANOVA was used. To compare OD for different tissue types and hormone treatments, two-factorial ANOVA was used. In both cases, Fisher’s protected least-significant-difference (PLSD) test was used for follow-up analysis to determine difference between groups (P < 0.01). In total, the quantitative immunohistochemical studies are based upon the analysis of 38 tissue recombinants and 23 uteri for PR, and 96 tissue recombinants for lactoferrin.

Preparation of Total RNA

Adult (approximately 90-day-old) female CD-1 mice were ovariectomized. Two weeks later, mice were given hormones as described above. All mice were killed 24 h after the last injection, and uteri were removed. Epithelium and stroma were separated as described above, and then the epithelium was flash-frozen in liquid N₂. Uteri from at least 2 mice were used for each treatment group in each experiment, and the entire experiment was repeated 3 times. Total RNA was prepared from frozen uterine and vaginal epithelial tissue by rapidly homogenizing the tissue in a guanidine isothiocyanate solution with a Polytron homogenizer (Brinkman Instruments, Westbury, NY). Total RNA was then isolated using the RNeasy Mini Kit (Qiagen, Chatsworth, CA). Purified total RNA was dissolved in diethylpyrocarbonate-treated water. Purity and concentration of the RNA was determined by UV (260/280) absorbance in a spectrophotometer.

Northern Blotting

For all Northern blots, equal amounts of uterine epithelial RNA from oil-, E₂-, and E₂+P₄-treated mice were electrophoresed on 1.5% agarose formaldehyde gels. The gels were blotted to nylon membranes, and the RNA was fixed to the membrane by UV cross-linking.
FIG. 1. ERα and PR immunostaining in the mouse uterus. Mature CD-1 female mice (approximately 90 days old) were ovariectomized and given hormone treatments 2 wk after ovariectomy as described in Materials and Methods. Oil (a and b), E2 (c and d), E2+P (shown as E2+P; e and f), and P (shown as P; g and h). Nuclei of ERα- (a, c, e, and g) and PR- (b, d, f, and h) positive cells stain brown; negative nuclei are blue because of the counterstain. ep, Epithelium; st, stroma; myo, myometrium.

The following cDNA probes were used in this study: 1) murine PR [21], a gift from Dr. G. Shyamala, Lawrence Berkeley Laboratory, Berkeley, CA, and 2) human 28S rRNA [22]. A fragment for the PR cDNA probe was isolated from the plasmid vector by restriction digestion and gel purification. Each fragment was labeled with 32P-dCTP using the multiprime DNA labeling system (Amersham, Arlington Heights, IL) and was used to probe the membrane.

All hybridizations were carried out in QuickHyb (Stratagene, La Jolla, CA) at 68°C. The hybridized membrane was washed, covered with plastic wrap, and exposed to Kodak X-omat x-ray film (Eastman Kodak, Rochester, NY) with intensifying screens. After hybridization with the PR cDNA probe, membranes were stripped of probe by incubation in 50% formamide at 65°C for 1 h; then the membrane was reprobed with labeled 28S rRNA cDNA probe, as described previously [23].

The mRNA transcript bands on the autoradiographs were scanned and quantitated using a computer-linked scanning laser densitometer and RFLPrint software (Pdi, Huntington Station, NY). The strongest band (the 6.9-kilobase [kb] band) for PR mRNA and the band for 28S rRNA were scanned, and the ratio of intensity of the 6.9-kb band to that of the 28S band (6.9 kb:28S) was calculated in each lane. In each blot, the ratio of 6.9 kb:28S in the oil-treated group was considered 100%. The data was analyzed by ANOVA and Fisher’s PLSD tests in the InStat statistical package (GraphPad Software Inc., San Diego, CA).

RESULTS

Regulation of PR by E2 and P4 in Uterine Epithelium

To study the effects of E2 and P4 on expression of PR in the uterus, mature wt female mice were ovariectomized, and 2 wk after ovariectomy all animals received injections of oil only, or E2 and/or P4. In all hormone treatment groups, ERα was detectable by immunohistochemistry in endometrial stroma, myometrium, luminal, and glandular epithelium (Fig. 1, a, c, e, and g).

In the uterus of ovariectomized oil-treated mice, both intensity of PR immunostaining and the number of PR-positive cells were low in the peripheral endometrial stroma and myometrium, but PR was strongly expressed in subepithelial endometrial stroma and in luminal and glandular uterine epithelium (Fig. 1b). E2 induced PR expression throughout the endometrial stroma and in the myometrium, while simultaneously down-regulating PR in the uterine epithelium (Fig. 1d). Pretreatment with P4 plus coadministration of E2 with E2 inhibited this down-regulation of epithelial PR induced by E2 (Fig. 1f). In E2 + P4-treated mice, both the percentage of PR-positive cells and PR signal intensity in uterine epithelium were higher than those in E2-treated mice (Fig. 1, d and f). Although E2-induced down-regulation of uterine epithelial PR was inhibited by P4, pretreatment and coadministration of P4 did not block E2-induced PR in endometrial stroma and myometrium of E2 + P4-treated mice (Fig. 1f). P4 alone had very little effect on uterine PR expression. Thus, in the P4-treated mice, the PR localization was very similar to that of oil-treated mice even though the PR immunostaining was slightly stronger than that of the oil-treated group in the subepithelial endometrial stroma (Fig. 1 h).

The effect of E2 and P4 on PR mRNA was analyzed by Northern blot in uterine epithelium (Fig. 2), and the results confirmed those of immunohistochemical studies. Uterine epithelial PR mRNA level was high in oil-treated mice and low in E2-treated mice. The PR mRNA level in uterine epithelium of E2 + P4-treated mice was intermediate between those of oil- and E2-treated mice.

The relative PR expression level in uterine epithelium was determined by measuring optical density of epithelial nuclei in PR immunostained sections. Morphometric analysis of PR expression in uterine epithelium showed clear differences among the hormone treatment groups (Fig. 2B, IHC). PR expression in uterine epithelium was significantly higher in the oil-treated group than in either the E2 group or the E2 + P4 group (P < 0.01), and was significantly higher in E2 + P4 group than in the E2 group (P < 0.01). Normalized densitometric data for uterine epithelial PR mRNA level was obtained by quantitating expression of the most abundant 6.9-kb PR transcript (Fig. 2B, Northern). PR mRNA expression in uterine epithelium in the oil-treated group, which was normalized to 100%, was high, and decreased to 25% in the E2-treated group. The reduction in uterine epithelial PR elicited by E2 was partially antagonized by P4 as shown by the smaller decrease in the 6.9-kb PR mRNA band in the E2 + P4 group compared to that in the group receiving E2 alone.

Role of Stromal PR in the Inhibitory Effect of P4 on E2-Induced Down-Regulation of Uterine Epithelial PR

Since PR is expressed in both uterine epithelium and stroma, we examined whether stromal PR was required to mediate the inhibitory effect of P4 on E2-induced down-regulation of uterine epithelial PR. Two types of uterine tissue recombinants were prepared with uterine epithelium (E) and stroma (S) from wt or PRKO mice: wt-S + wt-E and PRKO-S + wt-E. In the oil-treated (Fig. 3A, a and d) and E2-treated (Fig. 3A, b and c) groups, epithelial PR expression was essentially the same as that of host uterine tissue in both wt-S + wt-E and PRKO-S + wt-E tissue recombinants; epithelial PR was high in the oil-treated group and dramatically reduced in the E2-treated group. In contrast, P4 inhibition of E2-induced down-regulation of epithelial PR was observed only in wt-S + wt-E tissue recombinants. With E2 + P4, epithelial PR was strongly expressed in wt-S + wt-E tissue recombinants (Fig. 3A, e and f) indicating that the P4 had inhibited E2-induced down-regulation of epithelial PR. In contrast, PR was almost undetectable in PRKO-S + wt-E tissue recombinants treated with E2 + P4 (Fig. 3A, f) indicating that P4 had not inhibited E2-induced down-regulation of uterine epithelial PR. There was no observable difference in PR level between glandular and luminal epithelia (data not shown).

To show the change in epithelial PR level among different hormone treatment groups more clearly, the relative epithelial PR levels in uterine tissue recombinants were determined by measuring OD of epithelial nuclei in PR immunostained sections (Fig. 3B). In both wt-S + wt-E and PRKO-S + wt-E tissue recombinants, epithelial PR level was significantly higher in the oil-treated group than in the E2-treated and E2 + P4-treated groups (P < 0.01). In tissue recombinants composed with wt-S (wt-S + wt-E), epithelial PR level was significantly higher in the E2 + P4 group than...
in the E2 group, indicating that P4 was effective in partially inhibiting E2-induced down-regulation of uterine epithelial PR. In contrast, there was no significant difference in the epithelial PR level between E2 and E2+P4 groups (P < 0.01) in PRKO-S+wt-E tissue recombinants, indicating that P4 was not effective in inhibiting E2-induced down-regulation of uterine epithelial PR. This result clearly demonstrates that P4 action on uterine epithelial PR expression requires stromal PR. Uterine epithelial PR is not sufficient to block E2 action on uterine epithelial PR expression.

**Roles of Epithelial and Stromal PR in the Inhibitory Effect of P4 on E2-Induced Lactoferrin Expression**

To study the respective roles of epithelial and stromal PR in the P4 inhibitory effect on E2-induced lactoferrin expression, 4 types of tissue recombinants were prepared (wt-S+wt-E, wt-S+PRKO-E, PRKO-S+wt-E, and PRKO-S+PRKO-E). In all 4 types of tissue recombinants, lactoferrin was undetectable in the oil-treated group (Fig. 4A, a) and was dramatically induced in the E2-treated group (Fig. 4A, b). In all 4 types of tissue recombinants, lactoferrin staining was significantly higher in the E2-treated group than in the oil groups (P < 0.01; Fig. 4B). In addition, there were no significant differences (P < 0.01) in lactoferrin levels between the 4 types of tissue recombinants within the oil-treated group (Fig. 4B, columns with *) or the E2-treated groups. In wt-S+wt-E tissue recombinants, P4 completely inhibited E2-induced lactoferrin, and lactoferrin was undetectable (Fig. 4A, c). In wt-S+wt-E tissue recombinants, the lactoferrin level were identical for E2+P4-treated and oil-treated groups (Fig. 4B). In wt-S+PRKO-E tissue recombinants, P4 significantly reduced the level of E2-induced lactoferrin, but lactoferrin was still strongly detected, especially on apical side of the cytoplasm.

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**FIG. 3.** PR immunostaining in PRKO/wt uterine tissue recombinants. A) PR immunostaining in PRKO/wt uterine tissue recombinants. A) PR immunohistochemistry. Tissue recombinants were made with wt epithelium (wt-E) and wt stroma (wt-S) (a–c) or wt-E and PRKO stroma (ko-S) (d–f). Tissue recombinants were grafted under the renal capsule of female nude mouse hosts and grown for approximately a month; then all hosts were ovariectomized. Two weeks after ovariectomy, all hosts received hormone treatments as described in Materials and Methods. ko-S, PRKO-S. B) Epithelial PR level in PRKO/wt uterine tissue recombinants. Data are mean ± SEM of values and were statistically analyzed by two-factorial ANOVA and Fisher’s PLSD (P < 0.01).
FIG. 4. Lactoferrin immunostaining in PRKO/wt uterine tissue recombinants. Tissue recombinants were made with uterine E and S from wt and/or PRKO mice and were grafted under the renal capsule of female nude mice and grown for approximately a month. All hosts were then ovariectomized. Two weeks after ovariectomy, all hosts received daily injection of oil, E2 or E2+P, as described in Materials and Methods. A) Lactoferrin immunohistochemistry. FITC images were reversed, then superimposed on the brightfield image. B) Lactoferrin levels in PRKO/wt uterine tissue recombinants. Data are mean ± SEM of values. Data were statistically analyzed by two factorial-ANOVA and Fisher’s PLSD. Columns without *, significantly higher than those with * and ** (P < 0.01). **Significantly higher than * (P < 0.05).
The lactoferrin levels in wt-S+PRKO-E tissue recombinants were significantly higher in the E2+P4-treated group (Fig. 4B, a, column with **) than in the oil-treated group (P < 0.05), indicating that P4 partially inhibited E2 action. In contrast, in PRKO-S+wt-E (Fig. 4A, e) and PRKO-S+PRKO-E tissue recombinants (Fig. 4A, f), P4 did not down-regulate E2-induced lactoferrin expression, and lactoferrin levels in the E2+P4-treated group was identical to that of the E2-treated group (P < 0.01; Fig. 4B). These results clearly demonstrate that P4 inhibition of lactoferrin expression requires both epithelial and stromal PR. Uterine epithelial or stromal PR alone is not sufficient to block E2-induced lactoferrin expression.

**DISCUSSION**

In this study, we have shown for the first time that P4 inhibits E2-induced down-regulation of murine uterine epithelial PR in vivo, and that uterine stromal PR is essential for P4 to antagonize E2-induced down-regulation of epithelial PR. Our previous tissue recombinant studies using ERα knockout mice demonstrated that uterine epithelial PR is down-regulated by E2 through a paracrine mechanism mediated via stromal ERα [15]. Thus, the actions of both E2 and P4 on uterine epithelial PR involve paracrine mechanisms mediated by their respective steroid receptors in mouse uterine stroma. In both cases, epithelial ERα and PR are not sufficient to regulate mouse uterine epithelial PR in vivo. In a previous study, we demonstrated that epithelial ERα is likewise not required for E2-induced down-regulation of uterine epithelial PR [15]. Since expression of epithelial PR is the endpoint of the present study, we could not use PRKO mice to test whether epithelial PR is required for the inhibitory effect of P4 on E2-induced down-regulation of uterine epithelial PR. Nonetheless, analysis of the PRKO-S+wt-E tissue recombinant clearly shows for the first time that epithelial PR is not sufficient by itself to antagonize E2-induced down-regulation of uterine epithelial PR. Epithelial PR still may play some role because the binding of P4 to the epithelial PR may affect stability of the PR protein.

In the previous report, we demonstrated that E2 acts in vivo on murine uterine stroma to down-regulate uterine epithelial PR via paracrine mechanisms [15]. Many in vitro studies have shown that ERα and PR interact directly, and that occupied PR can act to repress transcription activity of ERα [24–27]. Given the scenario that E2 down-regulates uterine epithelial PR via stromal ERα and that P4 inhibits E2-induced down-regulation of epithelial PR through signals mediated via stromal PR, the inhibitory effect of P4 on E2-induced down-regulation of uterine epithelial PR could result from impaired synthesis of E2-induced paracrine factors. Alternatively, P4 itself may independently induce paracrine factors that antagonize the action of E2-induced paracrine mediators.

With regard to regulation of lactoferrin, our tissue recombinant study clearly showed that the full inhibition by P4 of E2-induced lactoferrin expression requires both epithelial and stromal PR. It has been believed that the PR level is critical in determining responsiveness of cells to P. Even though epithelial cells were derived from wild-type mice, epithelial PR level, and thus sensitivity of the entire tissue recombinant to P, should be exceedingly low in PRKO-S+wt-E tissue recombinants of the E2+P4-treated group, because P4 cannot oppose the action of E2 to down-regulate uterine epithelial PR because of an absence of stromal PR. This may explain why P4 did not inhibit E2-induced lactoferrin expression in PRKO-S+wt-E tissue recombinants. We also demonstrated that P4 can partially inhibit lactoferrin expression via stromal PR in wt-S+PRKO-E tissue recombinants. This result suggests the involvement of a paracrine mechanism in P4 regulation of lactoferrin expression, and agrees with our recent finding that both stromal and epithelial ERα are essential for induction of lactoferrin in the uterine epithelium by E2 [17]. We speculate that P4 acting through stromal PR inhibits lactoferrin expression by antagonizing E2-induced stromal signals, which are essential for lactoferrin expression in uterine epithelium.

Hormone target organs such as the uterus and vagina express ERα and PR in both epithelial and stromal cells. Recent studies using tissue recombinants composed of wild-type plus ERα knockout or wild-type plus PRKO epithelium and stroma are instrumental in defining the respective role of epithelial versus stromal ERα and PR in hormonal response. Through such studies, we have shown that E2-induced growth (DNA synthesis) of uterine, vaginal, and mammary epithelia is mediated through stromal ERα [20, 28, 29]. P4 inhibition of E2-induced uterine epithelial proliferation is likewise mediated through stromal PR [19]. Thus, in the mouse uterus, E2 and P4 regulate both epithelial proliferation and epithelial PR through paracrine mechanisms. These facts suggest the possibility that uterine epithelial PR expression is regulated through the cell cycle. However, a single injection of 125 ng E2 induces uterine epithelial proliferation but does not down-regulate uterine epithelial PR when assayed 18 h after hormone treatment [19]. Thus, uterine epithelial PR is probably not regulated by E2 and P4 through the cell cycle.

Regulation of murine uterine epithelial cell proliferation in vivo by E2 and P4 requires receptors in uterine stroma only and not in the epithelium. On the other hand, recent tissue recombinant studies showed that both stromal and epithelial ERα are essential for E2-induced cornification in vagina [28] and E2 induction of lactoferrin and C3 expression in uterus [17]. A role of epithelial PR in the regulation of lactoferrin by P4 in the mouse uterus was defined for the first time in this study by demonstrating that full inhibition of lactoferrin requires both epithelial and stromal PR. Further tissue recombination studies with PRKO and wt mice should define the respective functions of uterine epithelial and stromal PR in other actions of P4 in vivo. We emphasize that stromal PR should be required for most uterine epithelial functions that are regulated by a combination of E2 plus P4 since P4 modulates uterine epithelial levels of PR via stromal PR.

**REFERENCES**

6. Hild-Petito S, Verhage HG, Fazleabas AT. Immunocytochemical lo-


