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Authors

DeTomaso, Angela
Kim, Hyeyon
Shauh, Jacqueline
et al.

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Progesterone inactivation in decidual stromal cells: A mechanism for inflammation-induced parturition

Angela DeTomaso[a,b], Hyeyon Kim[b], Jacqueline Shauh[b], Anika Adulla[b], Sarah Zigo[b], Maya Ghoul[b], Pietro Presicce[c], Suhas G. Kallapur[c], Wendy Goodman[a], Tamara Tilburgs[d], Sing-Sing Way[d], David Hackney[b,e], John Moore[b,f], and Sam Mesiano[b,e,1]

Affiliations are included on p. 8.

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The process of human parturition involves inflammation at the interface where fetal chorion trophoblast cells interact with maternal decidual stromal (DS) cells and maternal immune cells in the decidua (endometrium of pregnancy). This study tested the hypothesis that inflammation at the chorion–decidua interface (CDI) induces labor by negating the capacity for progesterone (P4) to block labor and that this is mediated by inactivation of P4 in DS cells by aldo-keto reductase family 1 member C1 (AKR1C1). In human, Rhesus macaque, and mouse CDI, *AKR1C1* expression increased in association with term and preterm labor. In a human DS cell line and in explant cultures of term human fetal membranes containing the CDI, the prolabor inflammatory cytokine, interleukin-1ß (IL-1ß), and media conditioned by LPS-stimulated macrophages increased *AKR1C1* expression and coordinately reduced nuclear P4 levels and P4 responsiveness. Loss of P4 responsiveness was overcome by inhibition of AKR1C1 activity, inhibition of *AKR1C1* expression, and bypassing AKR1C1 activity with a P4 analog that is not metabolized by AKR1C1. Increased P4 activity in response to AKR1C1 inhibition was prevented by the P4 receptor antagonist RU486. Pharmacologic inhibition of AKR1C1 activity prevented parturition in a mouse model of inflammation-induced preterm parturition. The data suggest that inflammatory stimuli at the CDI drive labor by inducing AKR1C1-mediated P4 inactivation in DS cells and that inhibiting and/or bypassing of AKR1C1-mediated P4 inactivation is a plausible therapeutic strategy to mitigate the risk of inflammation-associated preterm birth.

pregnancy | parturition | inflammation | progesterone metabolism

Significance

Inflammation at the maternal–fetal interface induces parturition and is a major cause of preterm birth leading to neonatal morbidity and mortality. The mechanism by which inflammation induces parturition was examined. For most of pregnancy, the steroid hormone progesterone (P4) promotes uterine quiescence and blocks parturition, and loss of the P4 block induces parturition. Data from this study support the hypothesis that inflammatory stimuli induce parturition by causing a localized loss of P4 in maternal decidual stromal cells positioned at the maternal–fetal interface, and that this is mediated by the enzyme, aldo-keto reductase 1C1 (AKR1C1) that converts P4 to an inactive form. Importantly, the findings suggest that targeting AKR1C1 is a plausible approach to prevent inflammation-induced preterm birth.

The process of parturition (aka: labor and delivery) is triggered by inflammation in the uterine tissues. Inflammation in the amnion-chorion fetal membranes (FM), where fetal chorion trophoblast cells are in direct apposition with maternal uterine decidual stromal (DS) cells—the chorion-decidua interface (CDI)—is especially important. In women, term (37 to 40 completed weeks of gestation) parturition is typically associated with inflammation in the CDI, and preterm parturition (<37 completed weeks of gestation) is closely associated with chorioamnionitis, an overt and pathologic inflammation at the CDI (1–6). In animal models, various forms of experimentally induced inflammation trigger parturition (7–9). However, despite data clearly linking CDI inflammation to parturition, it remains unclear how inflammation transforms the uterus to the labor state. This study tested the hypothesis that inflammation at the CDI induces parturition by causing a localized withdrawal of progesterone (P4) in DS cells.

A conserved feature among viviparous species is that P4 acting via the nuclear P4 receptor (PR) in uterine cells, including DS cells at the CDI, induces a uterine phenotype conducive to the establishment and maintenance of pregnancy (10, 11). Clinical and animal studies show that loss of P4/PR signaling (e.g., by removal of the P4 source or by pharmacologic disruption of P4/PR signaling) alone triggers parturition at all stages of pregnancy (12–14). In most species, normal term parturition is triggered by a physiologically controlled decline in serum P4 levels (i.e., systemic P4 withdrawal). In humans, parturition occurs without systemic P4 withdrawal, and instead is thought to be triggered in uterine cells by loss of P4/PR signaling via altered PR transcriptional activity (15–21) and/or local inactivation of P4 (22, 23). Studies in myometrial cells suggest that proinflammatory stimuli increase expression of *AKR1C1,* that encodes aldo-ketoreductase (AKR) isoform 1C1 (AKR1C1) (23–25), a member of the AKR superfamily that shares sequence homology (~85%) with three other isoforms, AKR1C2, AKR1C3 and AKR1C4, and each with distinct preference for steroid substrates (26). Importantly, AKR1C1 is the most efficient at reducing biologically active P4 to the inactive 20α-hydroxyprogesterone, which is expected to inhibit P4/PR signaling (27). Thus, AKR1C1 may act as a P4 gatekeeper to induce an intracellular P4 withdrawal and

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[1]To whom correspondence may be addressed. Email: sxm188@case.edu.

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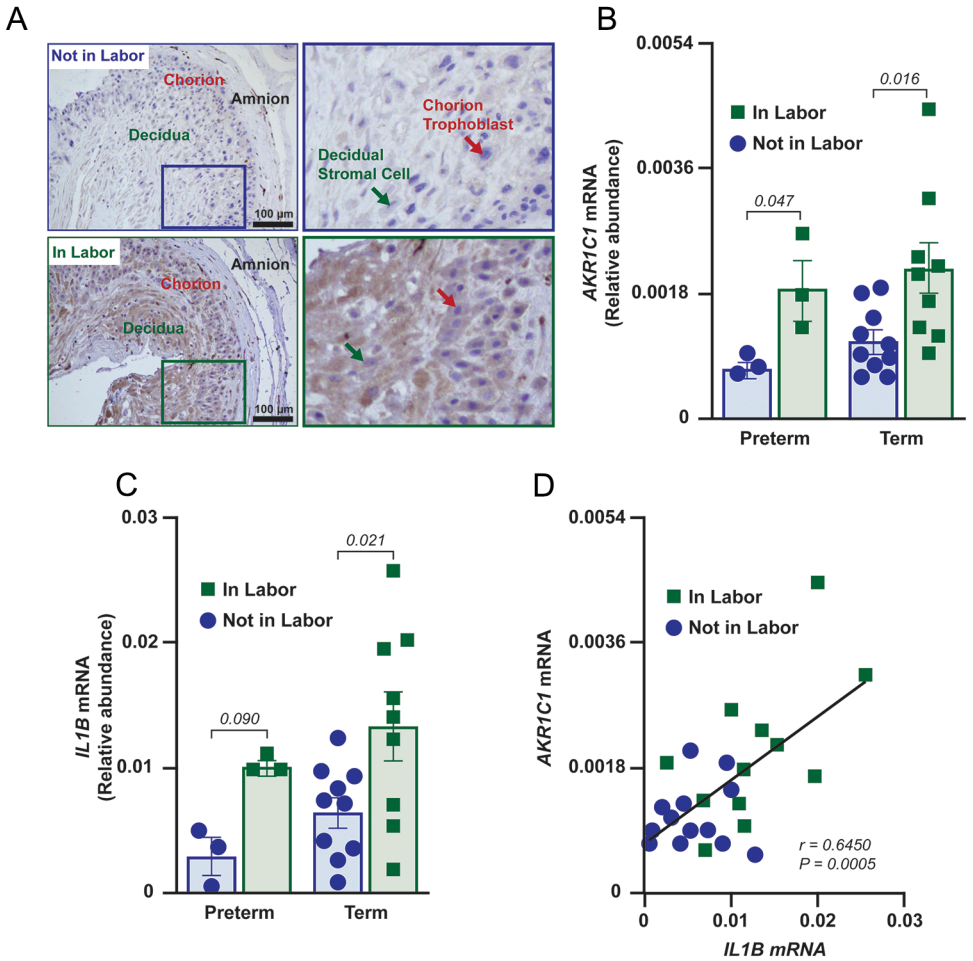

Fig. 1. AKR1C1 expression in human chorion-decidua increases in association with labor. (*A*) Immunohistochemical detection of AKR1C1 (brown staining) in the decidual compartment of the CDI at term (representative of N = 8). (*B*) *AKR1C1* and (*C*) *IL1B* expression in preterm (N = 3/group) and term [(N = 9 or 10/group) ± labor] CDI (mean ± SEM; *P* calculated by Student's *t* test). (*D*) Correlation (Pearson's) of *AKR1C1* and *IL1B* expression in human CDI (N = 25) (*P* calculated by Pearson's correlation test).

prevent PR activation. The present study examined the role and regulation of AKR1C1-mediated local P4 withdrawal in DS cells at the CDI in the context of normal and inflammation-induced parturition. The findings provide compelling evidence that prolabor inflammatory signals induce withdrawal of P4/PR signaling in DS cells via AKR1C1-mediated intracellular P4 metabolism. Importantly, the study provides plausibility for bypassing and/or inhibiting AKR1C1 activity as a therapeutic strategy to decrease the risk for inflammation-associated preterm birth, a major cause of neonate morbidity and mortality.

Results

AKR1C1 in DS Cells Increases in Association with Term and Preterm Labor. Our central hypothesis predicts that labor is associated with increased AKR1C1 expression in CDI tissue. AKR1C1 protein and *AKR1C1* expression (assessed by *AKR1C1* mRNA transcript abundance) were measured in FM (contains the CDI) collected from c-section deliveries performed before and during active labor at term (37 to 40 wk) and preterm (<37 wk). Immunoreactive AKR1C1 staining was detected in the nucleus and cytoplasm of multiple cell types within the CDI and was pronounced in DS cells. Staining intensity in DS cells was higher in laboring compared with prelabor tissue (Fig. 1*A*). Compared to prelabor tissue, the relative abundance of mRNA encoding AKR1C1 in CDI (amnion removed) was significantly higher in laboring tissue, and this was observed at term and preterm (Fig. 1*B*). As expected, labor at term and preterm was associated with increased expression of *IL1B* that encodes interleukin (IL)-1ß (IL-1ß), a major prolabor/proinflammatory cytokine in the CDI (Fig. 1*C*), supporting the concept that labor involves tissue-level inflammation at the CDI. The relative abundance of mRNAs encoding IL-1ß and AKR1C1 were positively correlated (Fig. 1*D*) in human CDI, suggesting a functional interaction between inflammation and *AKR1C1* expression in DS cells.

Prolabor Inflammatory Stimuli Increase *AKR1C1* Expression in DS Cells. Parturition is associated with and thought to be triggered by inflammation at the CDI (28, 29). In this context, IL-1ß is a major prolabor/proinflammatory cytokine that is up-regulated in uterine tissues, especially the decidual compartment, in association with parturition (30–32) and this was verified by our data (Fig. 1*D*). The functional interaction between IL-1ß and *AKR1C1* expression in DS cells was examined in term CDI and in a human DS cell line, hFM-DEC (*SI Appendix*, Fig. S1). IL-1ß increased *AKR1C1* expression in full thickness FM explants and in hFM-DEC cells (Fig. 2*A*). The data support a causal relationship whereby IL-1ß, produced as part of the local inflammatory milieu, induces *AKR1C1* expression in DS cells. This finding is consistent with other studies showing IL-1ß-induced *AKR1C1* expression in cervical stromal cells (33) and in bladder cancer cells (34).

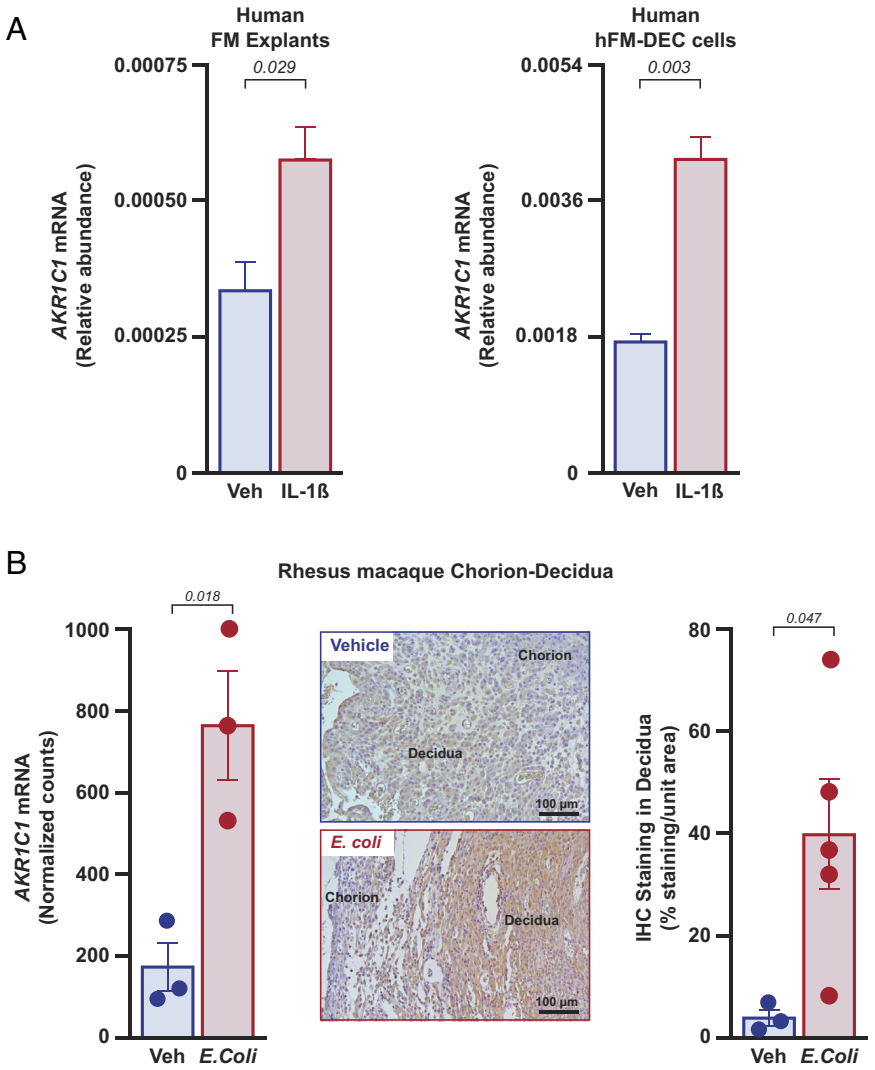

Fig. 2. Prolabor stimuli increase AKR1C1 in DS cells. (*A*) Effect of IL-1ß (1 ng/mL) or PBS for 12 h on the AKR1C1 expression in FM explants (*Left*) and hFM-DEC cells (*Right*) (mean ± SEM; *P* calculate by Student's *t* test). (*B*) AKR1C1 expression (*Left*) [normalized RNA reads obtained from GSE181054 (9)] (N = 3/group) and representative immunohistochemical localization (indicated by brown staining) of AKR1C1 in the CDI in Rhesus macaques 48 h after inoculation with *E. coli* or vehicle (Veh) into the amniotic fluid at 80% gestation (N = 3 or 5/group). Mean ± SEM; *P* calculated by Student's *t* test.

To further examine the causal relationship between AKR1C1 and inflammation in CDI tissue, *AKR1C1* expression was measured in CDI tissue from a Rhesus macaque model of preterm labor induced by intrauterine inoculation with live *E. coli* (1×10^6 colony-forming units) at 80% gestation (9). CDI tissue was collected 48 h after *E. coli* or saline inoculation. *E. coli* induced labor within 48 h, whereas animals treated with saline had no signs of labor. As with human CDI, AKR1C1 protein was detected in DS cells and AKR1C1 staining intensity and *AKR1C1* expression was increased in tissue from animals treated with *E. coli* (Fig. 2*B*).

Immune Cell Activation Increases *AKR1C1* Expression in DS Cells. Our core hypothesis predicts that proinflammatory signals derived from conditions such as intrauterine infection induce labor via AKR1C1-mediated localized P4 withdrawal. To test this hypothesis, we determined whether, lipopolysaccharide (LPS), the outer membrane component of gram-negative bacteria that activates the toll-like receptor 4-mediated proinflammatory pathways, affects AKR1C1 expression in DS cells. We found that in hFM-DEC cells, LPS did not affect *AKR1C1* expression (Fig. 3*A*) even though it had the predictable effect on increasing the expression of known proinflammatory genes (e.g., *CXCL8* and *IL6*). Unexpectedly, LPS did not increase *IL1B* expression in hFM-DEC cells. This outcome supports the hypothesis that in DS cells, expression of *AKR1C1* is primarily controlled by IL-1ß. The data also suggest that infection-derived signals (e.g., LPS) affect *AKR1C1* expression in DS cells indirectly. We therefore examined the effect of LPS in FM explants. In contrast to hFM-DEC cells, LPS increased *AKR1C1* expression in human FM explants (Fig. 3*B*) suggesting that within the CDI, other cell types play a role in mediating inflammation-induced *AKR1C1* expression in DS cells. As myeloid-derived cells are major producers of IL-1ß and other secreted prolabor factors at the CDI (35, 36), we tested the hypothesis that these immune cells produce proinflammatory cytokines in response to LPS that increase *AKR1C1* expression in DS cells. To do this, a monocyte-derived cell line, THP-1, was exposed to LPS or vehicle, and conditioned medium was added to hFM-DEC cells. Media from LPS-stimulated THP-1 cells increased *AKR1C1* expression in hFM-DEC cells (Fig. 3*C*). This effect was reduced but not completely blocked by anakinra, an IL-1 receptor antagonist (Fig. 3*D*), suggesting that in response to LPS, THP-1 cells produce IL-1ß and other soluble factors that stimulate *AKR1C1* expression in DS cells.

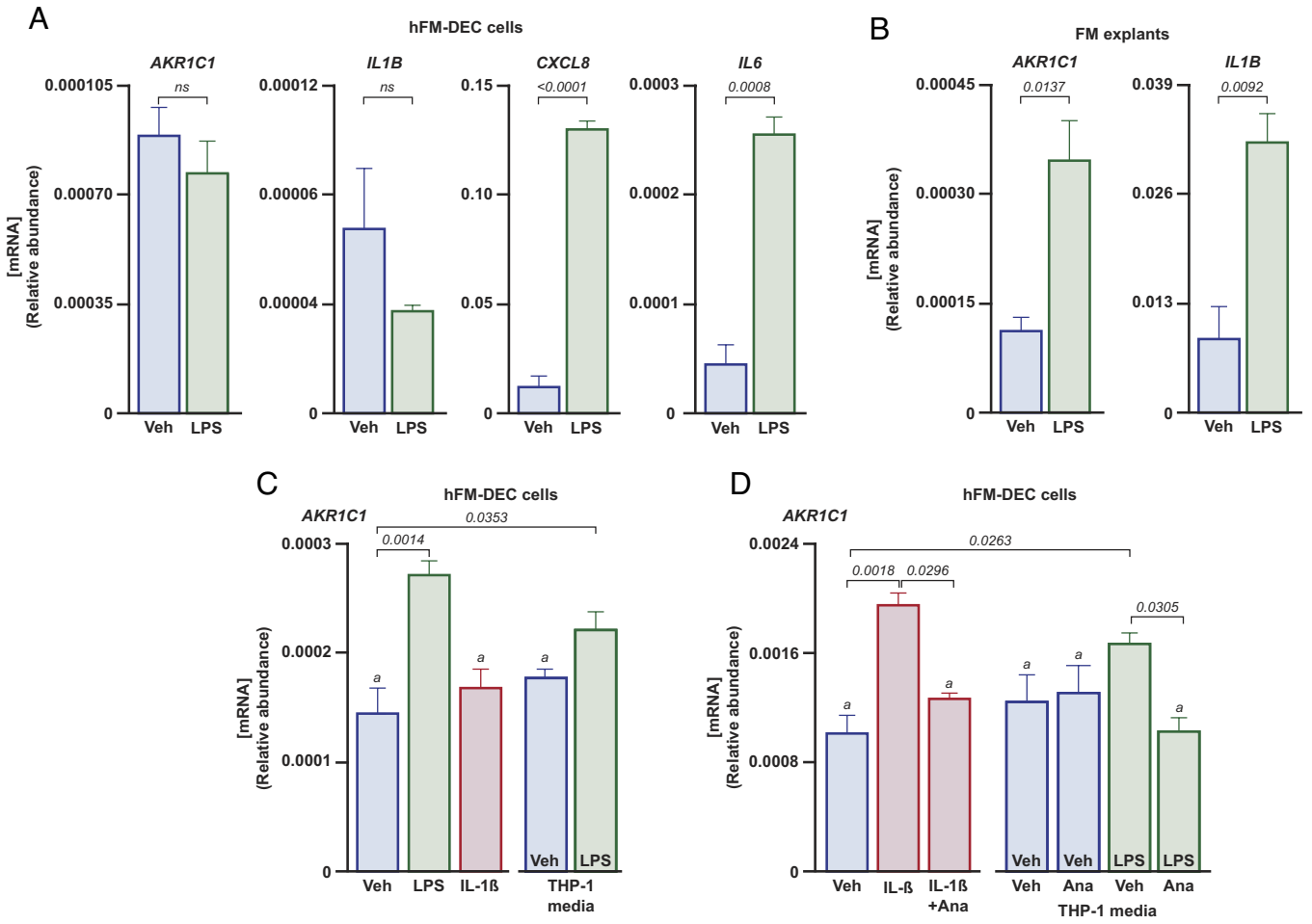

Fig. 3. LPS-stimulated immune cells increase *AKR1C1* expression in DS cells. (*A*) Effect of LPS (1 μg/mL) or PBS for 16 h on expression of *AKR1C1*, *IL1B*, *CXCL8,* and *IL6* in hFM-DEC cells. (*B*) Effect of LPS (1 ng/mL) or PBS for 16 h on *AKR1C1* expression in term, not in labor FM explants. (*C*) Effect of LPS (1 μg/mL), IL-1ß (1 ng/mL), and media from THP-1 cells exposed for 16 h to LPS (1 μg/mL) or vehicle (veh; PBS) on *AKR1C1* expression in hFM-DEC cells. (*D*) hFM-DEC cells were treated as in *C* ± the IL1R signaling antagonist, anakinra (ana; 2.5 μg/mL). Mean ± SEM; *P* values calculated by Student's *t* test (*A* and *B*) and one-way ANOVA (*C* and *D*). Groups that are not significantly different from one another ($P > 0.05$) are denoted by *a* (*C* and *D*).

AKR1C1 Inhibits P4/PR Signaling in DS Cells. Thus far, our data show that *AKR1C1* expression in DS cells is increased in association with the onset of labor and in response to prolabor/proinflammatory stimuli. Those findings, although supporting our central hypothesis, do not address the central tenet of the hypothesis: that increased AKR1C1 activity decreases P4/PR activity in DS cells. To test this, the effect of AKR1C1 on P4/PR transcriptional activity was determined in FM explants and in hFM-DEC cells exposed to IL-1ß. For this experiment, the readout for P4/PR activity was IL-1ß-induced expression of *CXCL8* (encodes IL-8) that is inhibited by P4/PR (18, 37) and expression of *IL15* that is increased by P4/PR (*SI Appendix*, Fig. S1). A further consideration for the experiments was that hFM-DEC cells are exposed to P4 and likewise, DS cells in FM explants are exposed to P4 produced by chorion trophoblast cells (*SI Appendix*, Fig. S2). As expected, in both models, IL-1ß induced a robust increase in *CXCL8* expression suggesting minimal basal P4/PR anti-inflammatory activity despite the presence of P4 and PR in both models. Importantly, the AKR1C1 inhibitor, 5-bromo-3-phenyl salicylic acid (BPSA) restored P4/PR anti-inflammatory activity as indicated by decreased IL-1ß-induced *CXCL8* expression in FM explants and hFM-DEC cells. This effect was dependent on P4/PR signaling since it was prevented by the PR antagonist RU486 (Fig. 4*A*). To further test the AKR1C1-mediated P4 metabolism hypothesis, FM explants were exposed to a P4 analog, R5020, that is not a substrate for AKR1C1 (38, 39). While endogenous P4 was ineffective, R5020 reduced IL-1ß-induced *CXCL8* expression in FM explants (Fig. 4*B*) and prevented IL-1ß-inhibition of *IL15* expression, a known P4/PR-responsive gene in DS cells (40, 41) (Fig. 4*C* and *SI Appendix*, Fig. S1). In hFM-DEC cells, BPSA also inhibited IL-1ß-induced *CXCL8* expression, and this also occurred when AKR1C1 was inhibited by RNA interference (RNAi) (Fig. 4*D* and *SI Appendix*, Fig. S3).

Our hypothesis posits that the metabolism of P4 by AKR1C1 imposes a barrier to P4 interaction with PRs in DS cells. This effect would be reflected by the amount of P4 sequestered to the nucleus via its interaction with PRs. Indeed, we found that IL-1ß significantly decreased the amount of P4 in the nucleus of hFM-DEC cells (Fig. 4*E*) and that the decrease was eliminated by *AKR1C1* RNAi knockdown. Taken together, these data support the hypothesis that IL-1ß-induced AKR1C1 metabolizes P4 in DS cells thereby causing a decrease in P4/PR transcriptional activity.

Inhibition of AKR1C1 Prevents Inflammation-Induced Parturition in Mice. A mouse model of inflammation-induced parturition was used to test the core prediction of our central hypothesis that proinflammatory stimuli induce parturition via AKR1C1-mediated P4 withdrawal. LPS induces parturition in mice when

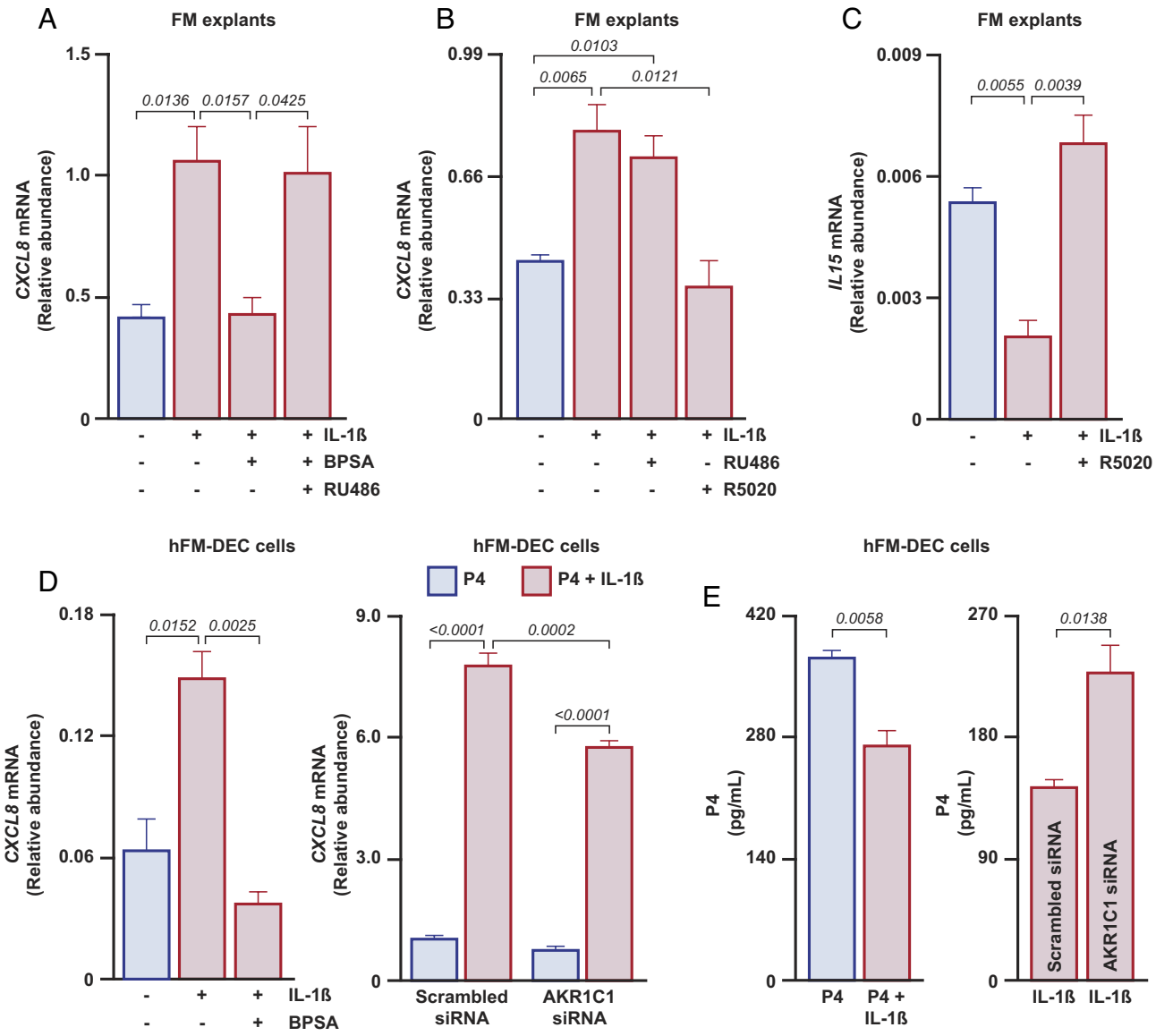

Fig. 4. AKR1C1 inhibits P4/PR signaling in DS cells. Effect of BPSA (100 nM), RU486 (50 nM), and R5020 (100 nM) on IL-1ß-induced *CXCL8* expression (*A* and *B*) and *IL15* expression (*C*) in FM explants (term not in labor). (*D*) Effect of AKR1C1 knockdown by RNAi (*SI Appendix*, Fig. S3) in hFM-DEC cells on IL-1ß-induced *CXCL8* expression. (*E*) Effect of IL-1ß (1 ng/mL for 16 h; *Left* or 22 h; *Right*) and AKR1C1 knockdown by RNAi (*Right*) on the level of P4 in nuclear lysates from hFM-DEC cells. Mean ± SEM; *P* values calculated by Student's *t* test.

administered either into the peritoneum or into the uterus (42, 43). We found that LPS administered into the peritoneum of mice at gestation day (GD)14.5 (term = GD19) induced parturition and significantly increased expression of *Akr1c18* (murine homolog of *AKR1C1*) and *Il1b* in the decidua (Fig. 5*A*). LPS induced *Il1b* expression at an earlier time point, compared to *Akr1c18*, suggesting that IL-1ß induction occurs upstream of *Akr1c18* expression. The role of Akr1c18 in parturition was assessed by determining the effect of BPSA on LPS-induced preterm labor (Fig. 5*B*). BPSA did not affect fetal weight or appear to cause fetal toxicity when administered to pregnant CD-1 mice during late gestation (*SI Appendix*, Fig. S4). Other studies confirmed that BPSA inhibits the 20α-hydroxysteroid dehydrogenase (inactivates P4) activity of murine Akr1c18 (44). Pregnant CD-1 mice at GD13.5 were treated with P4 ± BPSA. Previous studies suggest that in mice, LPS induces parturition secondary to systemic P4 withdrawal caused by increased *Akr1c18* expression in the maternal corpora lutea (CLs) (45–47). To account for this, mice were treated with P4 (1 mg) to ensure the LPS-induced parturition occurred without systemic P4 withdrawal. We found that P4 administration failed to prevent LPS-induced parturition, even though the dose of P4 administered was sufficient to prevent ovariectomy-induced parturition in mice of the same gestational age. Importantly, we found that BPSA prevented LPS-induced parturition with P4 supplementation. This finding is consistent with previous studies showing that R5020 (not metabolized by AKR1C1/Akr1c18) prevents LPS-induced parturition (48). Overall, the data support the hypothesis that inflammation induces parturition via AKR1C1-mediated P4 withdrawal in DS cells.

Discussion

The physiologic processes and hormonal interactions that transition the gravid uterus to the laboring state is a major unanswered question in obstetrics. This knowledge gap has impeded progress in clinical approaches to manage preterm parturition and the pathologies associated with premature birth. Clinical and animal

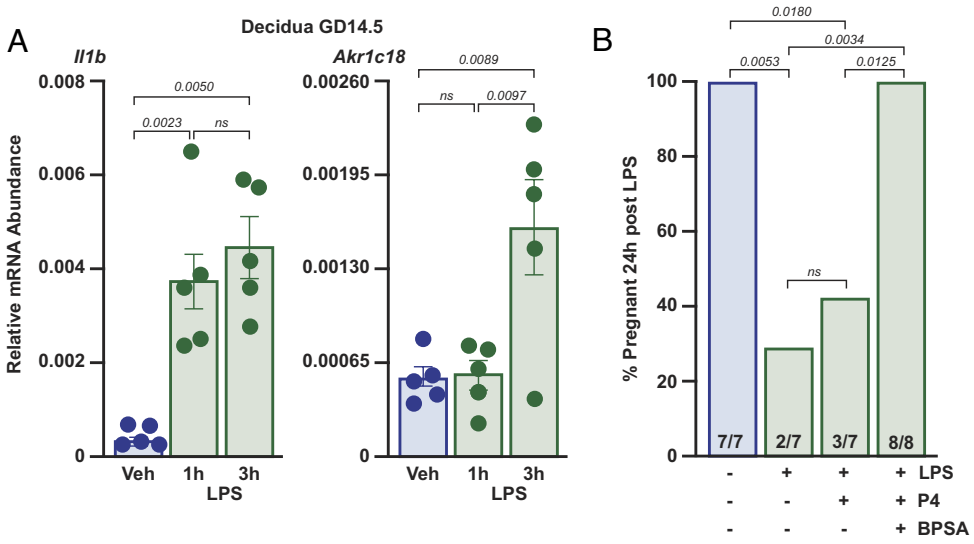

Fig. 5. Inhibition of AKR1C1 prevents inflammation-induced parturition in mice. Mice were treated with LPS on GD14.5. (*A*) Effect of LPS (100 μg) on *Il1b* (*Right*) and *Akr1c18* (*Left*) expression in the decidua of mice at 1 h and 3 h after treatment (N = 5/group). Mean ± SEM; *P* values calculated by one-way ANOVA. (*B*) Effect of BPSA on LPS-induced preterm birth (*Materials and Methods*); N = 7 or 8 mice/group; *P* values calculated by Chi-square test.

data show that parturition is an inflammatory event characterized by immune cell activation and infiltration into the uterine tissues, especially the decidua (49). Animal models clearly show that a proinflammatory challenge to the gravid uterus induces parturition, and clinical studies demonstrate a clear correlation between intrauterine infection/inflammation and the risk for preterm parturition (50). These observations favor the hypothesis that parturition, and particularly the pathophysiology of preterm parturition, involves and is induced by inflammatory stress in the uterine tissues, especially at the parietalis CDI.

Paracrine interactions between chorion trophoblast cells, DS cells, and resident immune cells in the CDI microenvironment are critical for the establishment and maintenance of pregnancy, with aberrations, especially pathologic inflammation, associated with adverse pregnancy outcomes such as preterm birth and preeclampsia. A key factor in the paracrine control of human pregnancy is P4 produced by chorion trophoblasts cells which acts on adjacent DS cells and resident maternal immune cells to promote immune quiescence (i.e., an anti-inflammatory effect) at the CDI. Although P4/PR signaling occurs in myometrial and cervical stromal cells, a recent study suggests that P4 withdrawal to induce labor originates in the decidua (51). In general, P4/PR signaling in DS cells is essential for pregnancy maintenance, presumably via anti-inflammatory effects, and loss or disruption of P4/PR transcriptional activity alone in DS cells is sufficient to trigger a proinflammatory cascade leading to parturition. This core propregnancy function of P4 in DS cells suggests that P4 withdrawal is a terminal event on which signals for parturition converge. In human pregnancy, the mechanism for P4 withdrawal is independent of circulating and local levels of P4—each of which is high before and during parturition—suggesting involvement of other mechanisms. In this context, our findings highlight the importance of AKR1C1-mediated local P4 metabolism in DS cells in the pathophysiology of parturition.

Our data show that labor at term and preterm in women is associated with increased *AKR1C1* expression in the FM parietalis CDI and specifically in DS cells; a finding consistent with previous studies (36, 52). Multiple studies in animal models show that inflammatory stress, induced artificially by administration of LPS (intrauterine or intraperitoneal), live *E. coli* (intrauterine), or IL-1ß (intrauterine) triggers parturition (7, 53–56). We examined the effect of inflammatory stimuli on *AKR1C1* expression in DS cells utilizing human FM explants and hFM-DEC cells as well as Rhesus macaque and mouse in vivo models of preterm labor. Overall, our data support the hypothesis that inflammatory stress induces *AKR1C1* expression in DS cells. Although the molecular mechanisms underlying the regulation of *AKR1C1* expression in DS cells have yet to be fully elucidated, our data show that IL-1ß is a key inducer.

Studies in myometrial cells suggest that P4/PR signaling blocks parturition, at least in part, by decreasing responsiveness to proinflammatory stimuli such as IL-1ß (as measured by expression of *CXCL8*, a well-characterized IL-1ß-dependent proinflammatory gene). In myometrial cells, P4 decreases IL-1ß responsiveness; however, in DS cells, P4 failed to decrease IL-1ß responsiveness, while R5020, a progestin that is not metabolized by AKR1C1, was effective. The AKR1C1 inhibitor, BPSA, inhibited IL-ß-induced *CXCL8* expression in a P4/PR-dependent manner. These observations directly link inhibition of AKR1C1 activity to P4/PR signaling. This concept is also supported by our finding that increased AKR1C1 correlates with decreased P4 retained in nuclear extracts and decreased P4/PR propregnancy activity, consistent with the hypothesis that AKR1C1-mediated metabolism of P4 leads to loss of PR activation. Importantly, the data suggest that AKR1C1 acts as gatekeeper for P4 access to PR in DS cells and that decreasing (e.g., BPSA treatment) or bypassing (e.g., R5020 treatment) AKR1C1 are plausible approaches to prevent, or at least decrease the risk for, inflammation-induced parturition (Fig. 6).

We determined the consequence of inhibiting AKR1C1 activity in a mouse model of inflammation-induced parturition. The finding that inhibition of AKR1C1 activity prevented LPS-induced parturition in the mouse is relevant because it reflects the importance of AKR1C1 in inflammation-induced parturition, and the plausibility of AKR1C1 inhibition therapy to reduce the risk of inflammation-associated preterm birth. Previously, we reported that LPS-induced parturition in mice is prevented by administration of R5020, and those findings demonstrate the possibility of bypassing P4 metabolism as a strategy to prevent inflammation-induced parturition (48). Our present findings further support the local P4 withdrawal hypothesis and suggest that inhibition of P4 metabolism by specific AKR1C1 inhibitors is an alternative therapeutic approach. The specificity of BPSA for AKR1C1 inhibition reflects

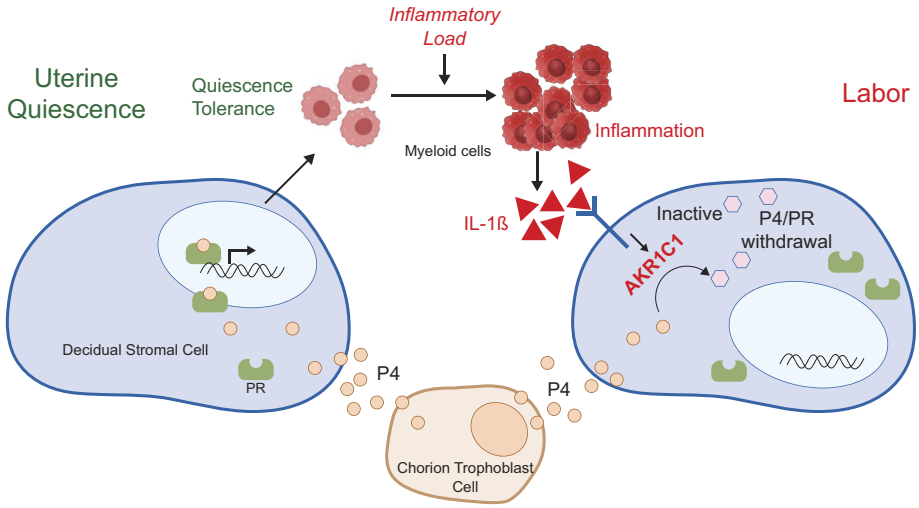

Fig. 6. Working model. We propose that for most of pregnancy P4/PR signaling in DS cells block labor in part by promoting IL-15-mediated tissue-level immune quiescence. In response to net inflammatory load (e.g., intrauterine infection; fetal development; maternal stress) immune cells at the CDI become activated leading to inflammation and the production of IL-1ß, which induces AKR1C1-mediated local P4/PR withdrawal (i.e., prevention of ligand activation of PRs) in DS cells leading to loss of the P4/PR block to labor and further amplification of tissue-level inflammation that transforms the adjacent myometrium and cervix to the labor state.

the importance of this AKR1C isoform in affecting P4 signaling during pregnancy. Currently, no effective therapies exist to mitigate the risk of preterm birth and especially preterm birth associated with intrauterine infection and/or chorioamnionitis. Our data demonstrated the plausibility of AKR1C1 inhibition and/or bypass as a unique approach to prevent or at least decrease the risk for preterm birth.

Materials and Methods

Cell Lines and Cell Culture. The hFM-DEC cell line (57, 58) was maintained in 1:1 Dulbecco's modified Eagle medium:nutrient mixture F12 (DMEM/F12) (Gibco cat no. 11330057) supplemented with glutamine (1% GlutaMAX; Gibco cat no. 35050061), penicillin/streptomycin (0.5% Gibco cat no. 15140122), and heat-inactivated (56 °C for 30 min) fetal bovine serum (5%; FBS; Gibco cat no. 10437028). hFM-DEC cells were induced to decidualize by exposure to 10 μM forskolin (Sigma Aldrich cat no. F3917), 100nM estradiol (E2) (Sigma Aldrich cat no. E2758), and 10 μM medroxyprogesterone acetate (MPA; Sigma Aldrich cat no. M1629) in phenol red-free DMEM/F-12 (Gibco cat no. 11039021) with supplements (as above) and 2.5% charcoal-stripped/heat inactivated FBS (Gibco cat no. 12676029) for 6 d (59–63). Decidualization was defined by altered morphology and increased expression of decidualization-specific genes *IGFBP1*, *IL15*, *HAND2*, *PRL*, and *PGR* (*SI Appendix*, Fig. S1). For experiments involving P4 quantitation, transfections, or exposure to test substances (e.g., BPSA, R5020), cells were first washed with PBS to remove serum and MPA and then equilibrated overnight in serum-free phenol red-free media with forskolin, E2 (100 nM), and P4 (250 nM), to mimic tissue levels during third trimester human pregnancy. For some experiments, AKR1C1 targeting siRNA (Silencer Select; Invitrogen cat no. s56843 and s56844) or nontargeting scrambled siRNA (Silencer Select; Invitrogen cat no. 13778030) was transfected into hFM-DEC cells using Lipofectamine RNAiMAX (Invitrogen).

The THP-1 monocyte cell line was maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco cat no. 61870036) supplemented with 15 mM HEPES (Gibco cat no. 15630080), 0.05 mM 2-mercaptoethanol (Gibco cat no. 31350010), 1 mM sodium pyruvate (Gibco cat no. 11360070), 0.5% penicillin/streptomycin, 10% heat-inactivated FBS. THP-1 cells were stimulated by exposure to LPS (1 μg/mL; derived from *E. coli* O111:B4, Sigma-Aldrich cat no. L2630) in hFM-DEC decidualization medium. Culture medium was collected the following day and centrifuged to pellet any nonadherent cells and debris. The supernatant (i.e., conditioned media) was collected and stored at −80 °C.

FM Tissue Collection and Explant Culture. Full thickness FM (containing the amnion, chorion, and attached decidua) was collected, with patient informed consent, from the placenta within 1 h of c-section delivery performed at University Hospitals in Cleveland (University Hospitals of Cleveland, Institutional Review Board approval 02-18-49). Study groups included women undergoing repeat c-section at term (37 to 40 wk gestation) or preterm (<37 wk gestation) who were not in labor or showed signs of labor (forceful contraction, cervix dilation ≥ 5 cm, and/or membrane rupture). Tissue was not collected from women with gestational diabetes, pre-eclampsia, fetal anomalies, or positive for Group B *Streptococcus*. FM was obtained from approximately the midpoint between the edge of the placental disk and the incision site and rinsed in PBS. A representative sample of full-thickness FM tissue was fixed in neutral-buffered formalin for future histologic analyses. For RNA analysis, amnion was pealed from the FM, and the remaining CDI was snap-frozen in liquid N2 and stored at −80 °C. For explant culture, full-thickness FM (5 × 5 mm pieces) from c-section deliveries performed at term with no clinical signs of labor was incubated at 37 °C for 6 h in DMEM/F-12 supplemented with 1% GlutaMAX and 0.5% penicillin/streptomycin prior to treatment.

mRNA Analysis. Total RNA was extracted using the acid-phenol method (TRIzol; Life Technologies cat no. 15596018) and quantified by OD260. 300 ng of total RNA was reverse transcribed using SuperScript II (Invitrogen cat no.18064). Quantitative reverse transcription PCR (qRT-PCR; primers shown in *SI Appendix*, Table S1) was performed as described (18) using SYBR green (Applied biosystems cat no. 4367659) to detect amplicon accumulation with each cycle. Relative mRNA abundance was determined using the ΔCT method with GAPDH mRNA as internal control. Relative abundance of specific mRNA transcripts was used as a surrogate for gene expression.

Immunohistochemistry. Sections (5 μm mounted on glass slides) of formalin-fixed paraffin-embedded FM were sectioned and subjected to immunostaining as previously described (18, 64). Sections were first subjected to antigen retrieval at 125 °C in using a citric acid-based antigen unmasking solution (Vector Labs cat no. H-3300) supplemented with 0.05% Tween-20. Sections were then incubated for 30 min at room temperature in blocking solution consisting of 2.5% normal horse serum (Vector Labs cat no. S-2000-20) in PBS then incubated overnight at 4 °C with mouse anti-AKR1C1 (Fitzgerald, cat no. 10R-1776) diluted 1:200 in antibody diluent (Cell Signaling Technology cat no. 8112L) or rabbit anti-AKR1C1 (GeneTex cat no. GTX105620) each in antibody diluent (Cell Signaling Technology cat no. 8112L). The following day, sections were washed and incubated with horseradish peroxidase-conjugated anti-rabbit (Cell Signaling Technology cat no. 8114S) secondary antibody for 30 min at room temperature and washed. Immunoreactive signal was then detected with diaminobenzidine substrate (Cell Signaling Technology cat no. 11725S) added for 3 min. Digital IHC images were analyzed using NIH-ImageJ to determine signal intensity (i.e., number of brown pixels) per section areas.

Immunoblotting. hFM-DEC cells were rinsed twice with ice-cold PBS and lysed in radioimmunoprecipitation assay (RIPA) buffer (Cell Signaling Technologies cat no. 9806) supplemented with protease and phosphatase inhibitors (Thermo Fisher cat no. 78441 and Roche cat no. 04693159001). Lysate was sonicated for 30 s and centrifuged at 12,000 ×g for 5 min at 4 °C. Protein concentration in the supernatant was measured using the BCA protein assay kit (Pierce cat no. 23227). Protein was diluted in denaturing loading buffer (Thermo Scientific cat no. J61337), heated for 5 min at 95 °C and subjected to denaturing polyacrylamide gel electrophoresis (Novex; Invitrogen, cat no. XP04200) and transferred from the gel to PVDF membrane (iBlot 2 dry transfer system; Invitrogen cat no. IB24002), and incubated in block buffer (ClearMilk; Pierce cat no. 37587) diluted in tris buffered saline containing 0.1% Tween-20 (TBST) at room temperature for 60 min with agitation. The PVDF membrane was then washed, incubated with mouse anti-AKR1C1 (Fitzgerald, cat no. 10R-1776) diluted 1:1,000 in blocking buffer overnight at 4 °C, washed, and incubated at room temperature for 1.5 h with horseradish peroxidase-conjugated anti-mouse IgG (Cell Signaling Technology cat no. 7076). The PVDF membrane was then washed with TBST and incubated with Radiance Q chemiluminescent detection reagent (Azure biosystems, cat. no. AC2101), and chemiluminescent signal was detected (Jess Simple Western instrument; Protein Simple).

Progesterone Assay. hFM-DEC were rinsed three times with PBS. Nuclear and cytoplasmic extracts were then prepared using the NE-PER kit (Thermo Fisher cat no. 78833) and nuclei were rinsed prior to lysis to minimize carry-over from the cytoplasmic fraction. P4 was measured in nuclear and cytoplasmic extracts by enzyme-linked immunosorbent assay (ELISA) (Enzo, cat no. ADI-900-011) according to the manufacturer's instructions.

Mouse Model for Inflammation-Induced Preterm Birth. CD-1 IGS outbred mice (Charles River Laboratories) were housed at Case Western Reserve University (CWRU) Animal Resource Center. Experiments were approved by the CWRU Institutional Animal Care and Use Committee (Protocol #2015-0104). Standard overnight timed matings were performed to produce pregnant mice with the appearance of a vaginal plug the following morning as gestation day (GD) 0.5 (term = GD19). To determine whether LPS induced *Akr1c18* expression in pregnant mice, PBS or LPS (100 μg) was injected intraperitoneally (IP) on GD 14.5. Tissues were harvested 1 or 3 h later and snap-frozen in liquid nitrogen until RNA extraction.

In some experiments, mice were treated with various test agents or an appropriate vehicle to determine the effects on LPS-induced parturition (see Figures). Mice were pretreated via SQ injection with vehicle or 50 μg BPSA dissolved in sesame oil 12 h prior to LPS treatment. The following morning, mice were injected IP with BPSA or vehicle dissolved in PBS and SQ with BPSA ± 500 μg P4 (AuroMedics Pharma LLC NDC 55150-306-10) dissolved in sesame oil. After 30 min, PBS or LPS (25 μg) challenge was administered via IP injection. This LPS dose was chosen due to the majority of animals delivering within 24 h. Additional IP injections of BPSA or vehicle occurred 2 and 4 h post-LPS injection. Eight hours later, IP injections of BPSA or vehicle and SQ injections of BPSA or vehicle ±250 μg of P4 were administered. This process was repeated every 8 h until 24 h post-LPS.

Statistical Analyses. Statistical analyses were conducted using Student's *t* test, ANOVA, or Chi-square test. Differences were considered statically significant if $P < 0.05$. Explant data shown are from one patient (three explants/condition) and are representative of three time-separated experiments, each from a unique term not in labor patient (n = 3/condition; N = 3). hFM-DEC cell data are from N = 3/condition and are representative of three time-separated experiments. All data are graphed as mean ± SEM.

Data, Materials, and Software Availability. All other data are included in the manuscript and/or *SI Appendix*. Previously published data were used for this work (Rhesus macaque transcriptome data and paraffin embedded tissues that were used in this study were generated as part of a previously published study: ref. 9.).

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Author affiliations: [a]Department of Pathology, Case Western Reserve University, Cleveland, OH 44106; [b]Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH 44106; [c]Department of Pediatrics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; [d]Cincinnati Children's Hospital, Center for Inflammation and Tolerance, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229; [e]Department of Obstetrics and Gynecology, University Hospitals, Cleveland, OH 44106; and [f]Department of Pediatrics, Case Western Reserve University, Cleveland, OH 44106

Author contributions: A.D. and S.M. designed research; A.D., H.K., J.S., A.A., S.Z., and M.G. performed research; A.D., P.P., S.G.K., W.G., T.T., S.-S.W., D.H., and J.M. contributed new reagents/analytic tools; A.D. and S.M. analyzed data; W.G., T.T., S.-S.W., and J.M. funding acquisition; D.H. clinical material acquisition; and A.D. and S.M. wrote the paper.

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