Localization and regulation of IL-1α in rat myometrium during late pregnancy and the postpartum period

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Received 3 January 2000; accepted in final form 25 October 2000

Melendez, J. Andres, James M. Vinci, John J. Jeffrey, and Brian D. Wilcox. Localization and regulation of IL-1α in rat myometrium during late pregnancy and the postpartum period. Am J Physiol Regulatory Integrative Comp Physiol 280: R879–R888, 2001.—Interleukin-1 (IL-1) has been implicated as a participant in preterm labor that is induced by bacterial infection. Previously, we showed that serotonin-induced production of IL-1α by myometrial smooth muscle cells in vitro is also essential for the synthesis of interstitial collagenase. It is therefore likely that IL-1α production in uterine tissues has implications for both the normal physiology of involution and for the pathophysiological mechanisms of preterm labor. The objective of this study was to characterize the serotonin-induced production of IL-1α by myometrial cultures in vitro and to assess the production of IL-1α and its relationship to collagenase production in vivo during pregnancy and the postpartum period. Immunohistochemistry demonstrated IL-1α protein in the nuclei and cytoplasm of serotonin-treated myometrial cells. IL-1α levels were decreased by treatment with progesterone or IL-1α receptor antagonist but were unaffected by lipopolysaccharide. Western analysis of myometrium from pregnant rats showed low levels of IL-1α during midpregnancy with increased concentrations at days 21 and 22 and postpartum. IL-1α mRNA levels also increased from days 15 to 22. Levels of mRNA for IL-1β also increased, although to a lesser degree than IL-1α. Both mRNAs decreased postpartum. Conversely, mRNA for interstitial collagenase was barely detectable at term but increased postpartum. Together, these data show that serotonin stimulates IL-1α production in vitro and indicate that normal myometrium from pregnant rats is an identifiable source of IL-1 during late pregnancy. The findings are consistent with the possibility that myometrial IL-1α participates in normal labor as well as the postpartum production of interstitial collagenase.

Interleukin-1 (IL-1) collectively refers to a gene family of three related cytokines that are involved in a variety of cellular processes. The family consists of the two agonist molecules, designated IL-1α and IL-1β, and a naturally occurring IL-receptor antagonist (IL-1ra). These cytokines are produced by numerous cell types in a diverse array of tissues and exert their biological activities by binding to the same membrane receptors. Although originally studied as proinflammatory mediators that are involved in immune responses, it is now known that these molecules exert a vast array of tissue- and cell-specific biological activities (9, 10).

The majority of the scientific interest in IL-1, both clinical and experimental, has been in the roles of these cytokines in proinflammatory settings, especially in response to infection. This interest is derived mainly from the original identification of IL-1 as an acute leukocytic product capable of inducing fever. In agreement with this proinflammatory role, there is a growing body of evidence linking the production of IL-1 with labor-inducing inflammatory processes that occur within the mammalian uterus (15, 31, 34). Both IL-1α and -1β, presumably the products of macrophages and lymphocytes, are detected in the amniotic fluid of women experiencing preterm labor due to uterine infection (17, 32). Similarly, the human decidua also produces IL-1 isoforms in response to bacterial products or infection (35). Once present, these cytokines have been shown to cause the synthesis and release of stimulatory prostaglandins, notably PGE₂ and PGF₂α, from the decidua and fetal membranes (24, 26, 30), an observation consistent with their participation in premature labor associated with infection. Causative support for the participation of IL-1 in preterm labor is given by the observation that exogenously administered IL-1 abruptly stimulates premature labor and delivery in pregnant mice (33).

Whereas the contribution of IL-1 to infection-induced preterm labor has been well studied, work from this laboratory has demonstrated that uterine myometrial cells themselves can produce IL-1 in response to hormonal stimulation by serotonin (13, 43). These studies were the first to demonstrate that IL-1 could be produced by cells derived from the myometrium in response to a hormone that is present and possibly increasing during late pregnancy (11). Interestingly, the induction of IL-1 mRNA in the myometrium is potently blocked by progesterone, an effect that may prevent the untimely induction of IL-1 before parturition (22, 43). More importantly, the production of IL-1...
by these cells is a crucial component of a normal non-inflammatory process that occurs within the uterus, that being the induction of interstitial collagenase synthesis that occurs after delivery (43). Although both IL-1α and -1β are produced by myometrial cells, we recently observed that IL-1α is the single IL-1 isoform that is specifically required for the production of collagenase (13). These studies are important because they demonstrate the necessity of the hormonal induction of a specific IL-1 isoform for a defined biological purpose in a normal uterine cell population in the absence of infection.

The requirement for serotonin-induced IL-1α in the production of collagenase raises the possibility that myometrial-derived IL-1 may be involved in other events occurring in the uterus, including the initiation of normal labor or in uninfected or otherwise idiopathic preterm labor. With this in mind, we are continuing to explore the mechanisms whereby serotonin and progesterone regulate IL-1 synthesis in myometrial smooth muscle cells. Both the pathological implications of IL-1-induced preterm labor and the requirement for IL-1 in collagenase production make the regulation of IL-1-induced preterm labor and the requirement for IL-1 in collagenase production a pivotal area for further research. In the studies presented here, we investigate the localization and regulation of IL-1 proteins in primary cultures of uterine smooth muscle cells and in the normal rat uterus during pregnancy and the postpartum period.

MATERIALS AND METHODS

Cell cultures. All experiments using cultured cells were conducted on primary isolates of rat uterine smooth muscle cells derived from 4-day postpartum uteri. Uteri were obtained, and cultures were prepared as described in detail previously (16). For all experiments, cells were plated in 75-cm² culture flasks and allowed to reach confluence in DMEM supplemented with 30 mM HEPES and 10% (vol/vol) fetal bovine serum (FBS) that had been adsorbed with dextrancoated charcoal to remove serotonin (CS-PBS (20, 38)). Other additions to medium are described in detail for each experiment.

Immunohistochemistry for IL-1α. Cell cultures for immunohistochemistry were plated directly onto 20 × 20-mm glass coverslips and treated as described in detail for each experiment (see RESULTS). Uterine tissue for immunohistochemistry was rapidly frozen in optimum cutting temperature embedding compound (Triangle Biomedical, Durham, NC) and stored at −70°C until cryosections (≈10 μm) were collected on gelatinized slides. All slides/cover slips were washed twice in PBS and then incubated in 3.7% formaldehyde at 37°C for 10 min. Adherent cells/tissue were washed and incubated for 5 min in 0.5% (vol/vol) Triton X-100, washed again, then incubated for 10 min in PBS containing 10 mg/ml glycine. Slides were again washed three times, then they were incubated at room temperature for 20 min with a 1:200 dilution of biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA). Slides were then washed twice with PBS, then incubated for 30 min with a 1:20 dilution of avidin-biotinylated peroxidase complex (Vector Laboratories). Slides were washed and incubated in 3,3′-diaminobenzidine peroxidase substrate for color development followed by distilled water. Photomicrographs were taken through an Olympus IMT-2 microscope on Kodak Royal Gold film.

Tissue extracts. For in vivo experiments, rats were euthanized by exposure to carbon dioxide, and the uterus was quickly removed. After the removal of the uterine contents, the endometrium was removed by gentle scraping with a fresh scalpel, and the myometrium was carefully dissected free from the underlying stroma, finely minced, and suspended in two volumes (wt/vol) of PBS containing 2 mM EDTA and 50 μg/ml protease inhibitors (Complete Mini, Boehringer-Mannheim, Mannheim, Germany). The tissue was then homogenized with a motor-driven homogenizer for 1 min at 4°C followed by sonication for 10 s at 4°C. Homogenates were centrifuged at 15,000 g for 15 min. Supernatants were aliquoted and stored at −70°C. Proteins in the pellet fractions were further solubilized in PBS containing 0.1% SDS, re-centrifuged, and supernatants were stored at −70°C.

Western blot analysis. Western blot analysis on tissue extracts was performed exactly as described previously (13, 22). The anti-rat IL-1α polyclonal antibody was obtained from Endogen (Cambridge, MA) and was used at a dilution of 1:200. The anti-rat IL-1β polyclonal antibody was obtained from R&D Systems (Minneapolis, MN) and was used at a dilution of 1:500. Alkaline phosphatase-conjugated secondary antibodies were from Sigma (St. Louis, MO).

RT-PCR analysis of IL-1α RNA. Total RNA from rat myometrial tissue was isolated using the RNA Isolator reagent (Genosys Biotechnologies, The Woodlands, TX) as described in detail previously (22). The cDNA was synthesized on 2 μg of RNA using Superscript II RT (Life Technologies, Gaithersburg, MD). Reactions (30 μl) contained 1× reverse transcriptase buffer (50 mM Tris-HCl, pH 8.3, 10 mM MgCl₂, 60 mM KCl, 10 mM 1,4-dithiothreitol), 0.5 μl of RNase inhibitor (Promega, Madison, WI), 0.5 μg/μl of oligo dT, 0.5 mM dNTPs, and 1.0 mg/ml BSA and were carried out for 1 h at 42°C. Oligonucleotide primers for PCR amplification were designed using the Oligo primer analysis software (National Biosciences, Plymouth, MN) and are shown in Table 1. All primers were designed to span introns so as to detect possible contamination of the RNA samples with genomic DNA. The PCR mixture (50 μl) consisted of 5 μl of cDNA from the RT reaction, 2.5 μl of each PCR primer (final concentration 1 μM), 2.5 mM dNTPs, 1× PCR buffer

Table 1. Primer pairs used for RT-PCR of mRNAs for rat IL-1α, IL-1β, and GAPDH

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>5′ Primer</th>
<th>3′ Primer</th>
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<tbody>
<tr>
<td>IL-1α</td>
<td>5′-CAGGGACAGAAGGAGCTCAAC-3′</td>
<td>5′-CAAAGAGGTTAATACATTACT-3′</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5′-CCTCTTCTTCTCTTCATCTTTG-3′</td>
<td>5′-CCGAGAGTCGGTGATGATAAC-3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-ATCTTTGCGATGCGGTTGAA-3′</td>
<td>5′-TGAGCGTCTGCGTGTGAC-3′</td>
</tr>
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All primer pairs were designed to span introns so that potentially contaminating genomic DNA would yield a product of increased molecular weight on agarose gel electrophoresis. IL, interleukin.
(50 mM KCl, 10 mM Tris·HCl, pH 8.3, 1.5 mM MgCl₂, 0.01% Gelatin), and 1.25 units of Taq DNA polymerase (Perkin-Elmer, Branchburg, NJ). Amplification was carried out for three cycles of 94°C for 60 s, 60°C for 2 min, 72°C for 2 min, followed by 27 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 15 s unless otherwise specified. After 30 cycles, the temperature was held at 72°C for 3 min. The products of PCR amplification were analyzed by electrophoresis on 1.5% agarose gels. Under these conditions, PCR amplification both of IL-1α and -1β remains in the linear range. RT-PCR analysis of GAPDH mRNA levels was modified to remain in the linear range of amplification by reducing the total number of PCR cycles from 30 to 18. The identity of a representative PCR sample for each amplified product was confirmed by bidirectional sequencing using the Taq DyeDeoxy terminator cycle-sequencing kit and an ABI model 310 DNA sequencer (Applied Biosystems).

**Northern blot analysis.** Total RNA from rat uterine tissues was isolated as described above for RT-PCR. RNA samples were electrophoresed on 1.5% agarose gels containing 0.66 M formaldehyde and blotted to nitrocellulose, as described in detail previously (8). Probes were radiolabeled with [α-32P]dCTP (3,000 Ci/mmol) using the Random Primer Plus Extension Labeling System (New England Nuclear, Boston, MA). Autoradiographic signals were quantified by video capture and digital image processing using the BioImage system (BioImage/Millipore, Ann Arbor, MI). The IL-1α probe is a 2-kb cDNA to mouse IL-1α cloned from mouse DBA/2 P88D1 cells [pmIL1aCDNA; (23)]. It was obtained through the American Type Culture Collection (Rockville, MD). The cDNA probe for rat collagenase is a 2.6-kb clone isolated from American Type Culture Collection (Rockville, MD). The cDNA probe for rat collagenase is a 2-kb cDNA to mouse IL-1α.

**RESULTS**

**Immunolocalization of IL-1α in myometrial smooth muscle cells.** Previous studies from this laboratory demonstrated that IL-1α mRNA production in myometrial cells is clearly induced by treatment with serotonin [5-hydroxytryptamine (5-HT)] alone or in combination with lipopolysaccharide (LPS) (19, 43). Recently, we also demonstrated that IL-1α protein is readily detected by Western blot analysis of myometrial smooth muscle cells (13, 22). To further characterize the localization and regulation of IL-1α in myometrial smooth muscle cells, we performed immunohistochemical detection of IL-1α protein. As depicted in Fig. 1, intense immunoreactive nuclear and cytoplasmic staining was observed in cells treated with either 5-HT alone or with a combination of 5-HT and LPS. In contrast, untreated cells showed only very faint cytoplasmic or nuclear staining. Because our prior studies showed that serotonin-induced IL-1α mRNA was blocked by treatment with progesterone or the IL-1α receptor antagonist, we also used immunohistochemistry to examine this regulation in more detail. As depicted in Fig. 2A, chronic treatment with either progesterone or the IL-1ra decreased both the nuclear and cytosolic levels of IL-1α immunoreactive protein induced by serotonin. In addition, progesterone and IL-1ra were able to block and reverse the serotonin-mediated induction of IL-1α protein (Fig. 2, B and C). These findings demonstrate that IL-1α protein is readily detected both in the nucleus and cytoplasm of serotonin-treated cells but is inhibited in the presence of either progesterone or IL-1ra.

**Western blot analysis of IL-1α and -1β in the pregnant and postpartum rat uterus.** Numerous studies both in humans and animals have implicated the involvement of IL-1 isoforms in endotoxin-induced abortion and in premature labor caused by infection. Our present findings, however, indicate that myometrial cells can produce IL-1α in response to hormonal stimuli in the absence of endotoxin or infection. For this reason, we next chose to characterize the levels of IL-1 protein in apparently normal, noninfected rat uterine tissue isolated from rats at various times during pregnancy. Our results are depicted in Fig. 3. Western blot analysis with an IL-1α-specific antibody demonstrated a faint immunoreactive band that was first observed on day 16 and was markedly increased in tissue isolated at day 21 of pregnancy and 2 days postpartum (Fig. 3A). IL-1α was only detected in cell supernatants and was not observed in the solubilized particulate fraction. Figure 3B shows the results of two other separate experiments conducted with cell supernatants from different animals. In these experiments, IL-1α protein was undetectable at days 5 and 10 of pregnancy but was readily detected at day 15. As in the previous experiment, IL-1α protein in tissue supernatants appeared to increase during late pregnancy and the postpartum period. Although an IL-1β-specific antibody could readily detect a purified IL-1β standard, no IL-1β was detected in any tissue homogenates at any stage of pregnancy. These data indicate that IL-1α is the predominant resident IL-1 isoform produced in the rat myometrium during normal pregnancy and show that levels of IL-1α increase before parturition.

**Immunolocalization of IL-1α in the pregnant and postpartum rat uterus.** We next used immunohistochemistry to further define the tissue distribution and temporal regulation of IL-1α in the nonpregnant, pregnant, and postpartum rat uterus (Fig. 4). IL-1α-specific immunoreactivity was weak or absent in the myometrium of nonpregnant uterus but was clearly observed in the myometrium of both pregnant and postpartum animals. This immunoreactivity was evident in tissues isolated at day 15 of pregnancy and was noticeably increased at day 21 of pregnancy and postpartum. In general, immunoreactive staining was much greater in the outer, longitudinal myometrium, especially at day 21 and postpartum. In contrast, IL-1α immunoreactivity in the endometrium and uterine glands was present in most tissues examined but was not observed to undergo noticeable changes during pregnancy. Control experiments that used normal rabbit serum in place of primary antibody (or secondary antibody alone) showed no immunoreactive staining. These data are in agreement with the results of Western blotting depicted in Fig. 3 and indicate that a significant portion of the uterine IL-1α detected during late pregnancy is produced by the longitudinal myometrium.
Characterization of IL-1 mRNA levels from rat myometrial tissue in vivo. We next sought to determine whether the production of IL-1 was correlated with changes in IL-1 mRNA and whether the production of IL-1α mRNA was altered before parturition. RT-PCR analysis showed that IL-1α mRNA was essentially undetectable in myometrium isolated on day 10 of pregnancy but steadily increased during late pregnancy (Fig. 5A). Peak levels of IL-1α mRNA were observed on days 21 and 22 of pregnancy, and a noticeable decrease in IL-1α mRNA occurred postpartum. IL-1β mRNA, on the other hand, was detectable early in pregnancy (day 10), also peaked at days 21 and 22, and was also detectable during the postpartum period. Overall, the relative levels of IL-1α mRNA were observed to undergo more significant changes than those

Fig. 1. Immunohistochemical detection of interleukin (IL)-1α in serotonin-treated and lipopolysaccharide (LPS)-treated rat myometrial smooth muscle cells in vitro. Primary cultures of rat myometrial smooth muscle cells were plated and allowed to reach confluence as described in MATERIALS AND METHODS. At confluence (~day 7), cells were treated as indicated until day 21 of culture, then they were analyzed for the presence of IL-1α protein as described. Light micrographs shown are representative of the appearance of cultures at ×10 and ×20 magnification after immunohistochemical detection. Concentrations used were serotonin (5-HT), 5 μM; LPS, 100 ng/ml.

Fig. 2. Effects of treatment with medroxyprogesterone acetate (Prog.) and IL-1-receptor antagonist (IL-1ra) on serotonin-stimulated production of IL-1α. A: primary cultures of myometrial smooth muscle cells were chronically treated as indicated from days 7 to 21 of culture as described in Fig. 1. B and C: cells were treated in either serotonin-containing (+5-HT) or serotonin-free (~5-HT) medium until day 21 of culture, then they received the indicated treatments for 48 h. Light micrographs shown are representative of the appearance of cultures at ×20 magnification after immunohistochemical detection of IL-1α. Concentrations used were 5-HT, 5 μM; Prog., 10⁻⁷ M; IL-1ra, 500 ng/ml.
eral, the qualitative pattern of expression of IL-1 mRNA increased slightly before parturition but was as early as postpartum rats showed detectable collagenase mRNA Northern blots of RNA isolated from pregnant and tissues were isolated from rat uteri on days 16 and 21 of pregnancy and the postpartum period.

The results of our first experiments, using primary cell cultures, demonstrate that IL-1α protein is indeed produced by rat myometrial cells and that the production of IL-1α protein mimics the previously reported regulation of its mRNA. IL-1α protein was undetectable in cultures treated without serotonin but was readily detected in its presence. Interestingly, simultaneous treatment with bacterial LPS had neither a positive nor negative effect on IL-1α levels, in agreement with our previous studies on IL-1α mRNA in similarly treated cells (43). This observation is of interest because of the well-studied implication of IL-1 isoforms as participants in preterm labor that is induced by bacterial infection. We previously showed that IL-1α mRNA can be stimulated by LPS but that LPS treatment is not necessary for its production in the presence of serotonin (43). Our current results show that the hormonal stimulation of IL-1α protein by serotonin also represents a maximal induction that is not augmented by the presence of bacterial products.

**DISCUSSION**

Recent work from this laboratory has focused on defining the hormonal regulation of IL-1α production in primary cultures of uterine smooth muscle cells. IL-1α is crucial in these cells as an intermediate in the production of interstitial collagenase (13). We first demonstrated that the ability of myometrial cells to produce IL-1α mRNA in vitro was dependent on stimulation by serotonin (43). Conversely, serotonin-stimulated increases in IL-1α mRNA were readily blocked by simultaneous treatment with either progesterone or the IL-1-receptor antagonist (22, 43). This work was intriguing in that it provided a paradigm whereby inflammatory cytokines could be produced for a specific purpose by resident uterine cells in a noninflammatory setting. Whereas IL-1 production by macrophages and lymphocytes has long been implicated as a participant in uterine pathologies caused by infection and ischemia, these data provided evidence that cytokines could have a distinctly physiological role when induced by hormonal stimuli in vitro. The data presented in this paper expand on this model in two important ways. First, we demonstrate that IL-1α protein can indeed be detected in myometrial smooth muscle cells in vitro and that its levels correlate with our previous studies on the regulation of its mRNA. Second, we provide evidence, for the first time, that the resident myometrial cells of the noninfected rat can be a source of IL-1 in vivo and that levels of this cytokine increase before the onset of labor and the postpartum increase in collagenase production.

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**Fig. 3. Western blot analysis of IL-1α and -1β in rat myometrial tissue during pregnancy and the postpartum period. A: myometrial tissues were isolated from rat uteri on days 16 and 21 of pregnancy and at 2 days postpartum as described in MATERIALS AND METHODS. Extracts from cell supernatants (s) and resolubilized pellet fractions (p) were analyzed separately. The blot was probed with a rabbit anti-rat IL-1α antibody as described in MATERIALS AND METHODS. B: two separate experiments in which only cell supernatants were analyzed from myometrial tissue isolated on days 5, 10, 15, 20, and 21 of pregnancy and at 2 days postpartum (P-P). Blots were probed with a rabbit anti-rat IL-1α antibody and with goat anti-rat IL-1β antibody as described in MATERIALS AND METHODS. Standards shown for size comparisons are purified mouse IL-1α and rat IL-1β.**

of IL-1β mRNA (Fig. 5B). These data demonstrate that the mRNAs for both IL-1α and -1β are significantly induced in the rat myometrium before parturition.

**Characterization of collagenase mRNA from rat myometrium in vivo.** Previous studies from this laboratory have focused on the ability of IL-1 isoforms to induce the production of interstitial collagenase from myometrial smooth muscle cells in vitro (13, 43). Our results presented here indicate that the rat myometrium in vivo can also synthesize IL-1α and that levels of IL-1α increase during late pregnancy. We, therefore, sought to determine whether collagenase mRNA is also produced by the rat myometrium in vivo. We used Northern blot analysis in these experiments because we have not yet undertaken a careful analysis of RT-PCR conditions that accurately represent quantitative changes in collagenase mRNA. We also reasoned that Northern blotting would represent a reasonable revalidation of the RT-PCR data obtained for IL-1α and -1β in Fig. 5. Northern blots of RNA isolated from pregnant and postpartum rats showed detectable collagenase mRNA as early as day 10 of pregnancy (Fig. 6). The amount of mRNA increased slightly before parturition but was markedly increased in the postpartum period. In general, the qualitative pattern of expression of IL-1α mRNA detected by Northern blotting was similar to that observed with RT-PCR with the exception that the method was notably less sensitive. We do not believe that differences in the two detection methods are due to differences in mRNA loading, because ethidium bromide staining of the gels before blotting demonstrated that all samples contained equivalent amounts of total RNA (data not shown).
The repressive actions of progesterone and IL-1ra on -1α mRNA have also been demonstrated in vitro (43). In the studies presented here, we show that these effects are also borne out at the level of IL-1α protein. Simultaneous treatment with either progesterone or IL-1ra inhibits or reverses the ability of serotonin to stimulate IL-1α-protein production. Therefore, in the presence of both a repressor and an inducer of IL-1 production, the repressor clearly dominates over expression of the protein. For IL-1ra, this repression indicates the importance of autocrine stimulation of IL-1 for the continued production of IL-1 protein by myometrial cells in vitro. Similarly, the repression mediated by progesterone could have profound implications on the production of IL-1 by the myometrium in vivo during mid- to late pregnancy, when progesterone is maintained at high levels. Previously, we demonstrated that the ability of progesterone to repress IL-1α mRNA production is receptor mediated and likely involves a protein-dependent increase in the instability of IL-1α mRNA (22). We are actively engaged in further defining this mechanism and determining whether a similar effect of progestins exists in vivo.

Immunohistochemical analysis of IL-1α in rat myometrial cell cultures demonstrated prominent serotonin-dependent increases in both nuclear and cytosolic staining. The reason why this staining pattern is distinctly heterogeneous, however, is not entirely clear. Prior immunohistochemical studies with anti-smooth muscle actin have demonstrated that our cultures are essentially entirely of smooth muscle origin (6). It is possible that the combined nuclear and cytosolic staining pattern is therefore linked to cell-cycle progression and, in a nonsynchronous culture system, results in a heterogeneous pattern of expression. This is in agreement with numerous other studies that have demonstrated cytoplasmic and nuclear localization of IL-1α (7, 25, 27, 42). Mizel et al. (27) demonstrated rapid internalization of 17 kDa IL-1α from the culture medium of murine fibroblasts or lymphoma cells and showed that it localized to the nucleus within 2–6 h. Other studies (25, 42) demonstrated that pro-IL-1α (28–35 kDa) also localizes to the nucleus, possibly due to a specific signal that is present within the first 115 amino acids of the IL-1α propiece. Although our studies did not differentiate pro-IL-1α from mature IL-1α in

![Image of immunolocalization of IL-1α in rat uterine tissues isolated during pregnancy and the postpartum period.](http://ajpregu.physiology.org/)
Each lane contains 5 μl of RT-PCR product from a separate animal. A: photographs of agarose gels showing the relative intensities of amplified cDNAs after RT-PCR. Each lane contains 5 μl of RT-PCR product from a separate animal. B: relative band intensities of the data shown in A after analysis using Molecular Dynamics IMAGEQUANT software. Bands for IL-1α or -1β were normalized to the level of GAPDH for each individual sample. The values shown are the means ± SE for each time period (days 21 and 22 are represented as 1 group). Statistically significant differences from day 10 (*P < 0.05, **P < 0.01) were determined using the 2-sided Student’s t-test.

Thus our findings indicate that IL-1α is predominantly located in a soluble intracellular pool within these cells. Indeed, most cells do not process and secrete IL-1α. Rather, the 17-kDa “processed” form is thought to derive from cleavage of the 30-kDa pro-IL-1α by extracellular proteases after its release from damaged cells (9, 18, 41). It is also interesting to note that we failed to detect the presence of IL-1β protein in these studies, although we previously demonstrated IL-1β mRNA in vitro (43). In many cell lines, pro-IL-1β is rapidly cleaved by the IL-1β-converting enzyme and secreted. This may explain our failure to detect IL-1β immunoreactive protein in crude myometrial extracts (9).

Western blot analysis of myometrial tissue from pregnant rats showed that IL-1α protein was present as early as day 15 of pregnancy and increased in concentration during late pregnancy and the postpartum period. This observation is intriguing because the initial rise in IL-1 concentration occurs during mid-pregnancy when progesterone concentrations should inhibit IL-1 expression. During this stage of pregnancy in the rat, the uterus is undergoing dramatic structural and morphological changes to accommodate the rapidly developing embryo. It is possible that at day 15, the production of IL-1α may be regulated by mechanisms that are, as yet, undefined or are restricted to defined regions of the uterus. In support of this hypothesis, preliminary studies from our laboratory indicate that IL-1α production at day 15 is more pronounced in the intervening regions of uterine tissue between implantation sites (unpublished observations). Interestingly, the IL-1α immunoreactive bands observed by Western blot also appeared to vary somewhat in molecular weight with an increase in size during late pregnancy. This increase was observed in three separate experiments (see Fig. 3). Despite this variation in size, the specificity of these antibody interactions was demonstrated by competition experiments using purified mouse IL-1α. When purified, IL-1α was added to the primary antibody solution; detection of all immunoreactive bands was abolished (data not shown).

A study by Hojo et al. (18) in rat bladder cancer cells also demonstrated the appearance of numerous IL-1α reactive bands at 10, 17, 28, and 31 kDa. Presumably, these proteins represented different cleavage products from mature pro-IL-1α. Although removal of the IL-1α pro-piece is generally thought to yield a fragment of 17 kDa, analysis of the IL-1α protein sequence also identifies a trypsin digestion site that would produce a 25- to 28-kDa fragment from the precursor molecule (28). The definitive identity of the different immunoreactive species of IL-1α in rat myometrium awaits microsequencing of the various fragments. Given that the molecular weight of IL-1α appeared to be consistently altered during late pregnancy, it will be interesting to assess whether these different forms of IL-1α have differing functions in the uterus.

The results of immunohistochemistry and Northern analysis of uterine tissue from pregnant rats indicate that IL-1α is predominantly localized to the outermost
longitudinal myometrium and that IL-1α levels appear to increase throughout late pregnancy. These results are in agreement with the results of Western blot analysis on dissected myometrial tissue and together indicate that the myometrium is a significant source of IL-1α during normal pregnancy in the rat. Previously, investigators definitively showed that the myometrium is also the principal source of uterine collagenase in vivo (6). Given that collagenase production in myometrial cells in vitro requires the presence of IL-1α, these results are also consistent with a role for IL-1α in the production of collagenase in vivo. Results from Northern blot analysis were consistent with this possibility and showed that increases in IL-1α mRNA at least precede the production of collagenase mRNA in myometrial tissue. Our Northern data are in contrast to those of Balbin et al. (4), who were unable to detect a signal for rat interstitial collagenase in the postpartum uterus. It is possible that these differences occurred because of rapid degradation of collagenase mRNA in the nuclease-rich environment of the postpartum uterus, an outcome that was avoided in the present study by rapid, immediate processing of uterine tissues for RNA extraction. Interestingly, concentrations of mRNA both for IL-1α and -1β were observed to decrease after delivery, possibly indicating that these molecules are indeed tightly regulated throughout late pregnancy and the postpartum period. Whether IL-1α regulates collagenase production in vivo remains to be established and is the focus of our current investigations.

Our previous studies on the regulation of IL-1α in vitro have concentrated on the upregulation of IL-1α transcription by serotonin (13, 19). In myometrial cell cultures, increased IL-1α protein and IL-1α gene expression occurs at serotonin concentrations that are found in the rat uterus, placenta, and maternal circulation during pregnancy (1, 14). Serotonin is also present at similar concentrations in the plasma of women during pregnancy and possibly increases before labor (11). Thus serotonin exists in vivo during pregnancy at levels that stimulate IL-1α expression in vitro. Given that a significant amount of serotonin circulates within platelet granules that are only released on platelet activation, locally elevated levels of serotonin are typically encountered in tissues during physiological or pathological responses that involve coagulation. This is mentioned because of the implication of placental bleeding, either gross or microscopic, in the genesis of preterm labor (36). Placental vascular abnormalities and ischemia have been noted to occur at significantly higher frequencies during preterm birth (37) and represent a subset of preterm deliveries that occur in the absence of bacterial colonization (3). It is therefore interesting to hypothesize that a subset of preterm deliveries might involve the induction of myometrial cytokines that are, in part, induced by elevated serotonin. We are currently at work on studies designed to test these hypotheses in human patients.

In summary, the results presented here show that resident myometrial cells of the uninfected rat uterus can be a significant source of cytokines during preg-
nancy. In doing so, this study is one of several that have recently shown that inflammatory cytokines are produced in uterine tissues in the absence of infection. For example, Steinborn et al. (40) showed that concentrations of IL-1α, IL-1β, and tumor necrosis factor-α were elevated in the cervicovaginal secretions of women during normal term delivery in the absence of infection. Other recent studies (2, 12, 39) also showed that decidual and placental IL-1β and -6 were readily detected in women in labor regardless of gestational age but were rarely detected in women undergoing cesarean section in the absence of labor. Taken together, these results suggest that common but temporally distinct pathways for both term and preterm labor may include increased levels of uterine-derived cytokines. In a normal, physiological setting, the production of IL-1 appears to be tightly regulated and to precede the onset of delivery. It is tempting to speculate that during at least a small percentage of preterm deliveries, increased serotonin may lead to the premature production of IL-1, a process that is designed to induce collagenase production and return the uterus to reproductive competence in the postpartum period.

Perspectives

The findings reported in this study provide evidence for an extended role of IL-1α in the tremendously complex processes involved in the normal termination of pregnancy and the onset of postpartum regression. Numerous studies have given rise to the notion that IL-1 plays a major role in preterm labor, particularly in the context of uterine infection. The possibility that the cytokine is involved in normal myometrial function during pregnancy has been suggested by numerous in vitro studies. These studies showed that the induction of the gene for interstitial collagenase, among others, is mediated by IL-1α in primary cultures of myometrial smooth muscle cells. Interstitial collagenase is a crucial enzyme for the removal of collagen of the uterus during postpartum involution. The current studies serve to bridge the gap between the in vitro findings and the situation in vivo; clearly, IL-1α levels rise during late pregnancy, preceding the appearance of interstitial collagenase in the tissue. Although a causal relationship has not been demonstrated, the temporal relationships are consistent with a role for the cytokine in collagenase induction. In any event, it seems clear from these studies that IL-1 plays a role in late pregnancy distinct from its more classical proinflammatory functions.

This work was supported by National Institutes of Health HD-32585 (to B. D. Wilcox), HD-05291 (to J. J. Jeffrey), and CA-77068 (to J. A. Melendez).

During the early stages of this work, J. A. Melendez was supported by a National Institute of Child Health and Human Development-sponsored supplement to HD-32585 for the support of underrepresented minorities in postdoctoral training.

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