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Disruption of Interleukin-18, but Not Interleukin-1, Increases Vulnerability to Preterm Delivery and Fetal Mortality after Intrauterine Inflammation

Xiaoyang Wang,^{*,†} Henrik Hagberg,^{*,‡}
Carina Mallard,^{*,‡} Changlian Zhu,^{*,†} Maj Hedtjärn,^{*}
Carl-Fredrik Tiger,[§] Kristina Eriksson,[¶]
Åsa Rosen,[‡] and Bo Jacobsson^{‡||}

From the Department of Physiology,^{*} Perinatal Center, and the Departments of Microbiology[§] and Rheumatology and Inflammation Research,[¶] Göteborg University, Göteborg, Sweden; the Department of Pediatrics,[†] the Department of Obstetrics and Gynecology,[‡] Perinatal Center, Sahlgrenska Academy, Göteborg, Sweden; The Third Affiliated Hospital of Zhengzhou University, Zhengzhou, People's Republic of China; and the North Atlantic Neuro-Epidemiology Alliances,^{||} University of Aarhus, Aarhus, Denmark

Preterm birth is a major contributor of adverse perinatal outcome. Clinical data suggest that an inflammatory response is important in the process leading to preterm labor. By using a recently introduced mouse model of localized intrauterine lipopolysaccharide-induced inflammation, the effect of interleukin (IL)-18 gene disruption and/or IL-18 neutralization as well as combined IL-1 α / β gene disruption on inflammation-induced fetal loss was investigated. The frequency of preterm fetal loss was significantly higher in IL-18 knockout mice (58.9%) and in mice administered IL-18-binding protein (59.7%) compared to wild-type controls (34.7%). The rate of fetal loss was not affected by IL-1 α / β gene deficiency (38.7%). Decreased IL-18 protein expression combined with elevated IL-12 protein expression in uterine tissue of IL-18 knockout mice and IL-18-binding protein-treated animals was noticed. These data demonstrate that preterm pregnancy loss in response to intrauterine inflammation was enhanced by disruption of the IL-18 gene and/or IL-18 neutralization, events that may relate to exaggerated Th1 responses because of an increased IL-12/IL-18 ratio. (*Am J Pathol* 2006, 169:967–976; DOI: 10.2353/ajpath.2006.050207)

Preterm birth affects 5 to 12% of all pregnancies and remains a major world-wide contributor of neonatal mor-

bidity, mortality, and long-term neurological handicap.^{1,2} Up to 30% of cases of spontaneous preterm birth are associated with intrauterine microbial colonization or histological evidence of chorioamnionitis. Approximately half of the cases of preterm labor and preterm prelabor rupture of membranes have intra-amniotic inflammation.^{3,4} The presence of intrauterine inflammation is not only a cause of preterm birth but is also associated with an increase in neonatal morbidity and mortality.⁵ Despite widespread use of preventive strategies, the rate of preterm birth has not decreased, with the survival of preterm infants having improved throughout the past several decades. As a consequence, the prevalence of long-term handicap attributed to preterm birth is increasing.⁶ Therefore, it is critical to elucidate the mechanisms and mediators that contribute to intrauterine inflammation-promoted preterm parturition and the child outcome.

Expression patterns of cytokines and other immunomodulatory proteins in the fetal membranes and decidua suggest that inflammatory activation occurs modestly with term labor but much more robustly in preterm delivery, particularly in the presence of intrauterine infection. Enhanced chemokine expression, particularly evident in deliveries involving an infected amniotic cavity, is presumably responsible for recruiting infiltrating leukocytes into the membranes, thereby amplifying the inflammatory process and hastening membrane rupture and delivery. In both humans and rodents, increased expression of proinflammatory cytokines and chemokines during infection-induced preterm labor has been recognized as a critical element of the hypothesized signaling cascade.^{5,7,8} Several members of the proinflammatory interleukin (IL)-1 family of cytokines (eg, IL-1 α , IL-1 β , and

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Address reprint requests to Xiaoyang Wang, M.D., Ph.D., Perinatal Center, Department of Physiology, Göteborg University, Box 432, S-405 30 Göteborg, Sweden. E-mail: xiaoyang.wang@fysiologi.gu.se.

IL-18) are supposed to play a key role in this process.^{8–11} IL-1 levels are elevated in the amniotic fluid of pregnancies complicated by intra-amniotic infection.⁹ IL-1 β has been found to be increased in a nonhuman primate model of infection-related preterm labor and to be capable of initiating preterm labor by up-regulating prostaglandin production, leading to myometrial contractions.¹² Moreover, administration of an IL-1 receptor antagonist inhibits IL-1-induced preterm labor in mice.¹³ Recent findings also suggest that polymorphisms in the IL-1 gene locus might be related to spontaneous preterm birth and abortion.^{14,15} However, the effect of IL-1 α combined with IL-1 β on preterm birth has not been studied.

IL-18 is a novel cytokine of the IL-1 family that is similar to IL-1 in terms of structure, processing, receptor complex, signal transduction pathway, and proinflammatory properties. Both IL-1 β and IL-18 are synthesized as inactive precursors that require cleavage by IL-1 β converting enzyme (caspase-1) to become fully bioactive.¹⁶ In addition, it was suggested that IL-18 shares functional similarities with IL-12, and both cytokines induce the production of interferon (IFN)- γ .¹⁷

In the present study, we used a recently developed mouse model of localized, lipopolysaccharide (LPS)-induced intrauterine inflammation¹⁸ to test the hypothesis that the deletion of the proinflammatory cytokine IL-1 α / β or IL-18 might reduce the incidence of preterm delivery. We adapted the model to C57BL/6 mice and tested both high and low doses of LPS exposure. The objectives of this study were to evaluate the effect of IL-18 gene disruption (IL-18 $^{-/-}$), IL-18 neutralization via IL-18-binding protein (IL-18BP), and combined IL-1 α / β gene disruption (IL-1 α β $^{-/-}$) in dams and their fetuses on inflammation-induced preterm birth and fetal loss and to investigate the related mechanisms and mediators that contribute to preterm parturition.

Materials and Methods

Animals

Time-mated pregnant C57BL/6 wild-type mice (originally obtained from Charles River Laboratories, Sulzfeld, Germany) and C57BL/6 mice lacking the gene for IL-18¹⁹ or IL-1 α β ²⁰ were bred under the same conditions at Experimental Biomedicine (Göteborg University, Göteborg, Sweden). The original C57BL/6 wild-type mice came from the same breeding colonies (founding strain) as the original C57BL/6 mice used for making the knockout mice. Both IL-1 α β - and IL-18-deficient mice were backcrossed to the C57BL/6 line for at least 10 generations before the experiments were started. For the experiments, IL-18 $^{-/-}$ females were impregnated by IL-18 $^{-/-}$ males, and IL-18 $^{+/+}$ females were impregnated by IL-18 $^{+/+}$ males. To ensure that the above IL-18 $^{-/-}$ animals did not differ in their response to LPS compared to IL-18 $^{-/-}$ animals derived from heterozygote breeding pairs, control experiments were performed in which homozygous (IL-18 $^{-/-}$) and heterozygous (IL-18 $^{+/-}$) mutant mice and wild-type

(IL-18 $^{+/+}$) littermate females were generated from heterozygous pairs. All animal experimentation was approved by the Ethical Committee of Göteborg (no. 290-2004).

Genotyping

The genotype of the IL-18 $^{-/-}$ and IL-1 α β $^{-/-}$ mice was determined by polymerase chain reaction (PCR) of genomic DNA obtained from both pregnant dam and pup tails.

DNA Preparation

The tail was digested with 400 μ l of lysis buffer (50 mmol/L Tris-HCl, pH 8.0, 100 mmol/L ethylenediaminetetraacetic acid, 100 mmol/L NaCl, 1% sodium dodecyl sulfate) containing 1 mg/ml proteinase K (Roche, Mannheim, Germany). After incubation at 60°C overnight, 200 μ l of 5 mol/L potassium acetate was added to the lysate, which was then thoroughly mixed and centrifuged at 10,000 $\times g$ for 20 minutes. The supernatant was transferred into a clean tube, and 800 μ l of 100% ethanol was added and mixed. After incubation at -20°C for 30 minutes, the DNA was pelleted by centrifugation at 10,000 $\times g$ for 25 minutes at 4°C. The pellet was washed once with 500 μ l of 75% ethanol. After removing all of the liquid, the pellet was left to dry at room temperature. The pellet of DNA was dissolved with 100 μ l of sterile water, and DNA concentration was determined.

PCR Amplification

IL-18 Wild-Type (Wt) and Knockout (KO)

Each PCR reaction (25 μ l) contained 1 μ l of genomic DNA, 0.2 mmol/L dNTP, 2.5 μ l 10 $\times$ PCR buffer (250 mmol/L Tris-HCl, pH 8.3, 375 mmol/L KCl, 15 mmol/L MgCl₂; Sigma, Stockholm, Sweden), 1 U of TaqDNA polymerase (Sigma), 1 μ mol/L of IL-18 common and IL-18 Wt primers, and 0.5 μ mol/L of IL-18 mutant primer. The PCR cycles were 94°C for 30 seconds, 67°C for 1 minute 30 seconds, and 74°C for 1 minute throughout 40 cycles. The following primers were used: IL-18 common, 5'-TTGCTGCACCTAGAGGTATGTACTGAC-3'; IL-18 Wt, 5'-TAATGGGTGGTCTTCTCATCTCTGTGT-3'; and IL-18 mutant, 5'-ATCGCCTTCTATCGCCTTCTTGACGAG-3'.

The IL-18 common and Wt primers detected the wild-type allele, which was ~300 bp, whereas the IL-18 common and IL-18 mutant primers detected the mutated allele, which was ~350 bp. Mice homozygous for the wild-type allele of IL-18 (IL-18 Wt) produced a single PCR band of 300 bp, mice homozygous for the mutated allele of IL-18 (IL-18 KO) produced a single PCR band of 350 bp, and mice heterozygous for the mutated allele of IL-18 (IL-18 het) produced double PCR bands of both 300 bp and 350 bp.

IL-1 α and IL-1 β Wt and KO

Each PCR (25 μ l) contained 2 to 4 μ l of genomic DNA, 0.8 mmol/L dNTP, 2.5 μ l of 10 $\times$ PCR buffer (250 mmol/L

Tris-HCl, pH 8.3, 375 mmol/L KCl, 15 mmol/L MgCl₂; Sigma), 2.5 U of *Taq*DNA polymerase (Sigma), and 1 μ mol/L of each primer. The PCR cycles were 94°C for 30 seconds, 63°C for 30 seconds, and 72°C for 1 minute 30 seconds throughout 40 cycles. The following primers were used: IL-1 β common, 5'-GCGAATGTGTCACAT-CTGCCACC-3'; IL-1 β Wt, 5'-GGTCAGTGTGTGGGTT-GCCTTATC-3'; IL-1 α mutant, 5'-GAGGTGCTGTTTCT-GGTCTTCACC-3'; IL-1 α common, 5'-CTTGCCCACT-GCAAAGGTCATG-3'; and IL-1 α Wt, 5'-CTTCTGCCTG-ACGAGCTTCATCAG-3'.

The IL-1 α common and Wt, IL-1 α common and IL-1 α mutant, IL-1 β common and Wt, IL-1 β common, and IL-1 α mutant primers detected the wild-type or mutated allele for IL-1 α or IL-1 β , respectively, which were all at ~1700 bp. Because the PCR product bands for the different genotypes of IL-1 β Wt/KO and IL-1 α Wt/KO were all of similar size, samples from the same animal had to be repeatedly run and each PCR reaction had to be performed in separate tubes. All primers were from CyberGene AB, Huddinge, Sweden. The PCR products were separated on a 1.5% agarose/0.5 $\times$ TBE gel containing ethidium bromide. A 100-bp ladder was used to verify the size of the PCR products. The gels were visualized using a LAS 1000 cooled charge-coupled device camera (Fujifilm, Tokyo, Japan).

Induction of Preterm Birth in C57BL/6 Mice

LPS (*Escherichia coli* 055:B5; Sigma) was injected into the uteri of pregnant mice as described elsewhere.¹⁸ Briefly, LPS was injected at a time corresponding to 79% of average gestation (ie, gestation day 15; C57BL/6 mice normally deliver pups on days 19 to 20 of gestation), which is an appropriate time to mimic late onset of human chorioamnionitis.^{6,21,22} Animals were anesthetized with isoflurane (5% for induction, 3.5% for maintenance) in nitrous oxide/oxygen (1:1) in an induction chamber. Laparotomy of the mouse lower abdomen was performed. The mouse uterus was exposed, and the number of fetuses in each horn was counted. LPS (250 μ g/mouse or 125 μ g/mouse) or saline of the same volume (100 μ l) was injected into the right uterine horn at a site between the lower two gestational sacs most proximal to the cervix. After the uterus was returned to the abdomen, the fascia and the skin were closed, and animals recovered in individual cages. The entire procedure lasted for less than 5 minutes per mouse. For mice treated with mouse IL-18-binding protein (IL-18BP, 122-BP; R&D Systems, Inc., Abingdon, UK), IL-18BP (15 μ g/mouse, ~0.5 mg/kg body weight) was administered intraperitoneally at the same time as LPS administration. Animals were observed every 4 to 6 hours for 48 hours for any signs of morbidity (piloerection, decreased movement), vaginal bleeding, and/or preterm delivery (at least one pup present in the cage or in the lower vagina within 48 hours of surgery). The number of live-born or dead pups delivered was recorded.

At 48 hours after LPS injection, all animals were laparotomized again, and the number of total fetuses (dead or

alive) left in the uterus horn were counted. Fetal deaths were identified by white discoloration, markedly smaller fetal size, and lack of blood flow in the umbilical cord. Uterine tissues from each group of animals including tissue from normal 17-day gestation pregnant mice were harvested and processed for immunoblotting and immunohistochemistry staining.

Immunoblotting

After harvesting, uterine tissue was washed in sterile saline, snap-frozen, and stored at -80°C. Tissue samples were homogenized by sonication in ice-cold 50 mmol/L Tris-HCl (pH 7.3) containing 5 mmol/L ethylenediaminetetraacetic acid and 0.5% protease inhibitor cocktail (P8340, Sigma), and samples were aliquoted and stored at -80°C. The protein concentration was determined according to Whitaker and Granum,²³ adapted for microplates. Samples were mixed with an equal volume of concentrated (3 $\times$) sodium dodecyl sulfate-polyacrylamide gel electrophoresis buffer and heated (96°C) for 5 minutes. Individual samples were run on 4 to 20% Tris-glycine gels (Novex, San Diego, CA) and transferred to reinforced nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). After blocking with 30 mmol/L Tris-HCl (pH 7.5), 100 mmol/L NaCl, and 0.1% Tween 20 (TBS-T) containing 5% fat-free milk powder for 1 hour at room temperature, the membranes were incubated with primary antibodies: goat anti-IL-18 (M-19; Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-actin (A2066, Sigma) at room temperature for 1 hour followed by an appropriate peroxidase-labeled, secondary antibody (Vector Laboratories, Burlingame, CA) for 30 minutes at room temperature. Immunoreactive species were visualized using the Super Signal Western Dura substrate (Pierce, Rockford, IL) and a LAS 1000-cooled charge-coupled device camera (Fujifilm). Immunoreactive bands were quantified using the Image Gauge software (Fujifilm).

Immunohistochemistry

Uterine tissue was washed in sterile saline and immersion-fixed in 5% buffered formaldehyde (Histofix; Histolab, Göteborg, Sweden) for 24 hours. After dehydration with graded ethanol and xylene, uterine tissues were paraffin-embedded. Uterus was cut into 6- μ m transverse sections across the lumen. Parallel sections were used for various stains, as described below. Antigen recovery was performed by heating the sections in 10 mmol/L boiling sodium citrate buffer (pH 6.0) for 10 minutes. Nonspecific binding was blocked for 30 minutes with 4% horse or goat serum (depending on the species used to raise the secondary antibody) in phosphate-buffered saline. The following primary antibodies were used: goat anti-IL-12A p35 (M-19, sc-9350; Santa Cruz Biotechnology), goat anti-IL-18 (M-19, sc-6179; Santa Cruz Biotechnology), and goat anti-mouse IL-18R α (AF856; R&D Systems, Minneapolis, MN). After incubating the primary antibodies for 60

Figure 1. The dose-dependent response of intrauterine LPS on preterm delivery (PTD) in mice. **A:** Preterm delivery rate. **B:** Incidence of fetuses delivered preterm (number of fetuses delivered preterm of total number of fetuses). **C:** Incidence of nonviable fetuses (number of dead fetuses retained in the uterus of total number of fetuses remaining in the uterus at 48 hours after intrauterine injection of LPS). LPS 250 (LPS 250 $\mu\text{g}/\text{mouse}$), LPS 125 (LPS 125 $\mu\text{g}/\text{mouse}$). *** $P < 0.001$ using Fisher's exact test.

minutes at room temperature, the appropriate, biotinylated secondary antibodies (all from Vector Laboratories) were added for 60 minutes at room temperature. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 5 minutes. Visualization was performed using Vectastain ABC Elite with 0.5 mg/ml 3,3'-diaminobenzidine enhanced with 15 mg/ml ammonium nickel sulfate, 2 mg/ml β -D glucose, 0.4 mg/ml ammonium chloride, and 0.01 mg/ml β -glucose oxidase (all from Sigma). Control experiments were performed by omission of the primary antibodies, substitution by goat serum (G9023; Sigma) for IL-12, and preabsorption of primary antibody with an excess (10 $\times$ and 100 $\times$) of

Figure 2. Representative genotyping results of the PCR analysis from IL-18 ($-/-$, $+/-$, $+/+$) and IL-1 $\alpha\beta$ ($+/+$, $-/-$) mouse tail DNA. The top panel shows representative PCR products of IL-1 $\alpha\beta$ and the bottom panel shows IL-18 genotypes. The molecular marker (M) for verifying the size of the PCR products was a 100-bp ladder. For IL-1 $\alpha\beta$ $^{-/-}$, the PCR products were only observed in the mutated allele for both IL-1 α and IL-1 β whereas absence of both IL-1 α and IL-1 β products indicated wild-type alleles. For IL-18 $^{-/-}$, the mutant allele-specific products were amplified by the primer sets of IL-18 common and IL-18 mutant, ~ 350 bp. The wild-type allele-specific products were detected from the wild-type allele-specific primer sets IL-18 common and wild-type, ~ 300 bp. Mice heterozygous for the mutated allele of IL-18 (IL-18 het) had products of both 300 bp and 350 bp.

blocking peptide (sc-6179 P; Santa Cruz Biotechnology) for IL-18.

Statistics

Fisher's exact test was used for comparing the differences between groups with respect to the number of fetuses delivered preterm and the number of nonviable fetuses. Spearman's rho method was used to test for trends of the starting time for the preterm delivery. Student's unpaired t -test was used to compare the average IL-18 immunoreactivity after densitometric quantification of individual samples after immunoblotting.

Results

Intrauterine Inflammation Induced Preterm Fetal Loss in a Dose-Dependent Manner in C57BL/6 Mice

Intrauterine injection of LPS (250 or 125 μg) evoked preterm birth in 100% (10 of 10) or 80% (8 of 10), respectively, of C57BL/6 dams within 26 hours (Figure 1A). All pups were nonviable, consistent with E15 to E16 of gestation. In contrast, none of the 10 mice injected with vehicle (saline) delivered preterm. The number of fetuses delivered preterm and the number of nonviable fetuses (dead fetuses retained in the uterine horn) were 51 of 70 (72.8%) and 14 of 19 (73.7%), respectively, in the group administered 250 μg of LPS, as compared to 25 of 75 (34.7%) and 27 of 49 (55.1%), respectively, in the group administered 125 μg of LPS (Figure 1, B and C). Lower doses of LPS (62.5 $\mu\text{g}/\text{mouse}$ or 15 $\mu\text{g}/\text{mouse}$), however, did not cause preterm delivery but evoked intrauterine fetal death (14 of 23, 60.8%) as evaluated at 48 hours after LPS administration.

Figure 3. Effect of IL-18 and IL-1αβ on the rate of preterm delivery (PTD). The incidence of fetuses delivered preterm (A, C) and incidence of nonviable fetuses (B, D) at 48 hours after LPS administration are shown. **A and B:** LPS dosage 250 μg/mouse for wild-type mice (Wt) and IL-1αβ^{-/-} mice. **C and D:** LPS dosage 125 μg/mouse for wild-type mice (LPS 125, *n* = 10), IL-1αβ^{-/-} mice (*n* = 5), IL-18BP co-treated wild-type mice (IL-18BP, *n* = 19), and IL-18^{-/-} mice (*n* = 10). ***P* < 0.01, ****P* < 0.001 using Fisher's exact test.

IL-1αβ Gene Disruption Did Not Affect Preterm Delivery

To investigate the role of IL-1 in preterm parturition, mice lacking both IL-1α and IL-1β (IL-1αβ^{-/-}) were used. The genotyping of IL-1αβ^{-/-} mice is shown in Figure 2. IL-1αβ^{-/-} mice injected with saline did not display any sign of preterm delivery. The number of fetuses delivered preterm and the number of nonviable fetuses at 48 hours after LPS administration (LPS 250 μg) did not significantly differ in IL-1αβ^{-/-} mice compared to wild-type mice. The number of preterm fetuses and nonviable fetuses was 24 of 33 (72.7%) and 51 of 70 (72.8%) (*P* = 1.00, Figure 3A), respectively, in IL-1αβ^{-/-} mice compared to nine of nine (100%) and 14 of 19 (73.7%) (*P* = 0.14, Figure 3B), respectively, in wild-type mice. To exclude the possibility that an excessively high dose of LPS might conceal a dependency on the IL-1αβ genotype, we decreased the LPS dosage to 125 μg/mouse. Again, there was no significant difference in the number of fetuses delivered preterm or the number of nonviable fetuses in IL-1αβ^{-/-} mice compared with the wild-type mice. The number of fetuses delivered preterm was 12 of 31 (38.7%) and 26 of 75 (34.7%)

in IL-1αβ^{-/-} mice versus wild-type mice (*P* = 0.66, Figure 3C), respectively. The number of nonviable fetuses was 11 of 19 (57.9%) and 27 of 49 (55.1%) in IL-1αβ^{-/-} mice versus wild-type mice (*P* = 1.0, Figure 3D), respectively.

IL-18 Deficiency Increased Preterm Delivery and Fetal Mortality

Next, to test the hypothesis that deletion of IL-18, a novel member of the IL-1 family of proinflammatory cytokines, reduces preterm delivery after intrauterine LPS administration, we compared IL-18 gene-deficient and wild-type mice. The mutated IL-18 allele was detected in all IL-18^{-/-} mice as shown in Figure 2. Intrauterine saline injection did not induce preterm delivery in IL-18^{-/-} mice as expected. But unexpectedly, when IL-18^{-/-} mice were injected with 125 μg/mouse LPS, we found a significantly increased number of fetuses that were delivered preterm and an increased number of nonviable fetuses compared with wild-type mice. The number of fetuses delivered preterm was 43 of 73 (58.9%) in IL-18^{-/-} mice versus 26 of 75 (34.7%) in

Figure 6. IL-12, IL-18, and IL-18R immunoreactivity in uterine tissue sections. Representative immunohistochemistry staining by using the IL-12 (A, D, G, J, M), IL-18 (B, E, H, K, N), and IL-18R (C, F, I, L, O) antibodies based on different mouse strains and treatments. Cont, normal wild-type mouse at 17 days of gestation (A–C); LPS 125, wild-type mouse with LPS treatment (D–F); IL-18BP, wild-type mouse with LPS and IL-18BP co-treatment (G–I); IL-18^{-/-}, IL-18^{-/-} mice with LPS treatment (J–L); IL-1αβ^{-/-}, IL-1αβ^{-/-} mice with LPS treatment (M–O). LPS dosage was 125 μg/mouse in all of the groups. Original magnifications, ×10.

found (Figure 6K). IL-1αβ^{-/-} mice treated with LPS displayed a moderate IL-18 staining not different from LPS controls (Figure 6N).

Because there is a strong interaction between IL-12 and IL-18 with respect to IFN-γ production, we examined the IL-12 immunoreactivity in parallel uterine sections. Very weak IL-12 immunoreactivity was detected in uterine tissue of normal control (Figure 6A) and LPS-treated (Figure 6D) wild-type mice. Although the

IL-18BP co-treatment mice (Figure 6G), LPS-treated IL-18^{-/-} mice (Figure 6J), and IL-1αβ^{-/-} mice (Figure 6M) displayed a moderately increased IL-12 staining in the decidua compared with control animals. IL-18R immunoreactivity (Figure 6, C, F, I, L, and O) was found primarily in decidua and to some extent in the smooth muscle cells of controls but the expression was not affected by LPS administration in wild-type, gene-deficient, or IL-18BP-treated mice.

Discussion

In the present study, we found that LPS induced preterm fetal loss in C57BL/6 mice in a dose-dependent manner, which agrees with the previous report in heat-killed *E. coli*-induced preterm delivery in mice.²⁴ The preterm pregnancy loss in response to intrauterine LPS administration in C57BL/6 mice was not affected by the combined disruption of the IL-1 α and β genes though, unexpectedly, enhanced by the disruption of the IL-18 gene and/or IL-18 neutralization. The enhanced preterm delivery rate was associated with decreased IL-18 in combination with increased IL-12 protein expression in the uterus. In contrast, increased expression of IL-12 alone, as found in the uterine tissue of IL-1 α $\beta^{-/-}$ mice, was not accompanied by an increased rate of preterm delivery.

Clinical studies have demonstrated that IL-1 levels increase in both amniotic fluid and gestational tissues toward parturition and are significantly elevated during labor.^{25,26} Furthermore, infusion of IL-1 α and tumor necrosis factor (TNF)- α into the amniotic cavity of rabbits,²⁷ or systemic administration of IL-1 β to mice,¹³ induced preterm delivery. In the present study, we found that the preterm pregnancy loss in response to intrauterine LPS administration in C57BL/6 mice was not affected by the combined disruption of the IL-1 α and β genes, suggesting that IL-1 may not play an essential role in the pathophysiology of labor during infection in this model. This is in agreement with previous studies that demonstrate that IL-1 β knockout²⁸ and IL-1 receptor knockout²⁹ mice are not more prone to preterm pregnancy loss than wild-type controls.

IL-18 is an 18-kd proinflammatory cytokine structurally and functionally related to IL-1 β .³⁰ IL-18BP decreases endogenous IL-18 activity by abolishing IL-18 induction of IFN- γ .³¹ Four isoforms of IL-18BP have been described in humans and two in mice.³² Isoform a, used in the present study, has the highest affinity for IL-18.³³ We found that both IL-18 gene disruption and IL-18BP co-treatment enhanced the sensitivity to LPS-induced preterm delivery. Whether it is the mother or the fetus that plays the most important role in the effect of IL-18 needs to be further addressed in future studies.

In addition to the high sensitivity of IL-18 $^{-/-}$ mice to LPS-induced preterm delivery, we found that no single fetus was alive 48 hours after LPS administration in IL-18 $^{-/-}$ mice. This is probably attributable to the high susceptibility of the IL-18 $^{-/-}$ mice to LPS-induced inflammation. Kohmura and colleagues³⁴ suggested that although LPS injected into mothers could pass through placenta to fetuses, LPS-induced intrauterine fetal death depends mainly on maternal sensitivity to LPS. It is suggested that IL-18 is important in the host defense against severe infections via induction of other cytokines, effector cells, and molecules.³⁵ The survival time of IL-18/IL-12 double-knockout mice was significantly shorter than that of IL-12 single-knockout mice in response to *Mycobacterium tuberculosis* infections, suggesting the possible contribution of IL-18 in host protection.³⁶ Thus, we speculate that the absence of IL-18 may increase the mouse vul-

Table 1. Scheme of Possible LPS-Induced Preterm Delivery Mechanisms Related to the IL-18/IL-12 Balance

Mouse strain/ treatment	IL-18	IL-12	Outcomes
Wild type	↑	No change	PTD
IL-1 α $\beta^{-/-}$	No change	↑	PTD
IL-18BP	↓	↑	Enhanced PTD
IL-18 $^{-/-}$	↓	↑	Enhanced PTD

nerability against LPS-induced inflammation. However, the underlying mechanisms of IL-18 deletion-provoked intrauterine fetal death need to be further investigated.

There are a limited number of studies regarding the expression and localization of IL-12, IL-18, and IL-18R in gestational tissues.^{37,38} In the present study, we found that IL-12, IL-18, and IL-18R were expressed in the decidua and smooth muscle cells, in agreement with previous reports. LPS increased the level of IL-18 in the mouse uterine tissue as found by both immunohistochemistry and immunoblotting compared with vehicle controls. Interestingly, in the IL-18 $^{-/-}$ mouse and IL-18BP-treated mouse uterine tissue in which absence or decreased levels of IL-18 were noted, we observed an increased level of IL-12 not seen in wild-type controls. This is consistent with reports from humans that showed the presence of IL-12 immunoexpression in intrauterine tissues in connection with preterm delivery.^{39,40}

Because IL-18 shares functional similarities with IL-12,³⁰ it is possible that IL-12 is increased in the absence of IL-18 as part of a compensatory response. However, a study from Kinjo and colleagues³⁶ showed that serum IL-12 levels were significantly lower in IL-18-deficient mice than in wild-type mice after infection with *M. tuberculosis*. This suggests that the balance between IL-18 and IL-12 may not be simply associated with compensation. Other closely related cytokines such as IL-15,⁴¹ IL-12 family new members IL-23 and IL-27¹⁷ may be involved in the host response as well.

In the uterus of IL-1 α $\beta^{-/-}$ mice exposed to LPS, we found increased levels of IL-12 whereas IL-18 was unchanged. Taken together (Table 1), increased levels of IL-18 (as seen in wild-type mice) or IL-12 (as found in IL-1 α $\beta^{-/-}$ mice) was associated with unaltered response to LPS. In contrast, increased IL-12 combined with decreased IL-18 was associated with increased susceptibility to LPS. These results are consistent with the view that the IL-12/IL-18 balance determines Th1/Th2 responses.^{37,39,42} A high IL-12 level combined with low IL-18 level increases Th1 predominance and would ultimately increase the proinflammatory response in preterm delivery. Indeed, such a delicate relationship between IL-18 and IL-12 has also been proposed to be important in severe preeclampsia.⁴³

Hypothetically, a proper balance between these two cytokines is necessary to both contest infection and protect the host from immune-based injury. IL-18 is likely to have different effects alone or if in combination with high levels of IL-12 in preterm delivery and pregnancy. Zourbas and colleagues⁴⁴ have undertaken a systematic

study of the expression of IL-12, IL-18, and other cytokines in the uterine tissues throughout pregnancy. They found that these cytokines have specific location and distinct sequential expression patterns, suggesting that they have important regulatory functions. Absence or excessively high levels of one or both of these cytokines may have immediate (implantation failures, abortion) or delayed (preeclampsia) consequences.^{38,42,45,46}

The different reactions in IL-1 $\alpha\beta^{-/-}$ and IL-18 $^{-/-}$ mice to LPS-induced preterm delivery might also relate to TNF- α . LPS induces TNF- α , which is strongly involved in preterm labor and pregnancy loss (for a summary, see a recent review by Romero et al⁴⁷). Indeed, an elevated level of TNF- α has been noted in the uterine wall and fetal membranes in mouse models of heat-killed *E. coli*-induced preterm delivery.^{24,48} Previous work from Sakao and colleagues⁴⁹ showed that LPS challenge increased serum TNF- α level in IL-18 $^{-/-}$ mice compared with those of wild-type mice, thus indicating that IL-18 is a negative regulator for TNF- α during sepsis. On the other hand, Reznikov and colleagues²⁸ observed reductions of TNF- α in IL-1 $\beta^{-/-}$ mice compared to IL-1 $\beta^{+/+}$ mice. We speculate that the different reaction of IL-1 $\alpha\beta$ and IL-18 deficiency to the LPS-induced preterm delivery might relate to different levels of and response to TNF- α after LPS in these mice. However, the precise role of TNF- α in LPS-induced preterm delivery in these mice remains to be defined.

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