Published in final edited form as:

Am J Obstet Gynecol. 2009 March ; 200(3): 308.e1–308.e9. doi:10.1016/j.ajog.2008.10.043.

Evidence for participation of uterine natural killer cells in the mechanisms responsible for spontaneous preterm labor and delivery

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Abstract

Objective—The purpose of this study was to determine in a mouse model whether uterine natural killer (uNK) cell cytotoxic activation induces infection/inflammation-associated preterm labor and delivery.

Study design—Wild type or interleukin (IL)-10^{-/-} mice were injected intraperitoneally with lipopolysaccharide on gestational day 14. Mice were either killed for collection of uteroplacental tissue, spleen, and serum or allowed to deliver. Uteroplacental tissue was used for histology and characterization of uNK cells.

Results—Low-dose lipopolysaccharide treatment triggered preterm labor and delivery in IL-10^{-/-}, but not wild type mice, in a manner independent of progesterone levels. Preterm labor and delivery in IL-10^{-/-} mice was associated with an increased number and placental infiltration of cytotoxic uNK cells and placental cell death. Depletion of NK cells or tumor necrosis factor (TNF) α neutralization in these mice restored term delivery. Furthermore, TNF α neutralization prevented uNK cell infiltration and placental cell apoptosis.

Conclusion—The uNK cell-TNF α -IL-10 axis plays an important role in the genesis of infection/inflammation-induced preterm labor/delivery.

Keywords

cytokines; inflammation; preterm birth; uterine natural killer cells

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INTRODUCTION

Perinatology Research Branch is the leading cause of perinatal morbidity and mortality.^{1–3} Of pregnancies in the United States, > 12% result in preterm birth, a rate that has increased over the years.⁴ The etiology of preterm labor and delivery is complex. A causal association has been proven in the case of intrauterine infection and inflammation. Despite this knowledge, no effective treatment or prevention for preterm birth is yet available.^{5–7} Normal parturition involves the activation of a complex inflammatory cascade.^{8–13} Premature activation of this cascade by intrauterine infection leads to spontaneous preterm labor and delivery.⁶ Thus, an understanding of the mechanisms linking intrauterine infection and inflammation to premature birth is crucial.

Preterm labor involves a change in the intrauterine cytokine milieu.¹⁴ We have demonstrated that the onset of spontaneous labor at term is associated with the down-regulation of placental interleukin (IL)-10, a major anti-inflammatory cytokine, and up-regulation of the pro-inflammatory cytokines IL-1 β and tumor necrosis factor (TNF) α .¹⁵ Down-regulation of IL-10 may be required for labor initiation as it is a potent inhibitor of various proinflammatory factors associated with parturition.^{16–18} We have shown that IL-10 production is reduced in placental explants from women with preterm labor.^{19,20} Conversely, an increase in messenger RNA expression and production for such proinflammatory cytokines as IL-1 β and TNF α has been demonstrated in preterm birth.^{9,21–26} Thus, these findings suggest that IL-10 may act as a temporal regulator of gestational length. Studies of pregnancy in IL-10^{–/–} (knock out) mice and other animals demonstrate that IL-10 regulates the effects of endogenous inflammatory insults, protecting pregnancy.^{27–30} However, the underlying mechanisms associating IL-10 deficiency to inflammation-induced preterm birth remain poorly understood.

Local innate immunity at the maternal-fetal interface is crucial for fetal development and immunoprotection, but its potential involvement in pregnancy-associated disorders has received little attention. During decidualization in human beings, uterine natural killer (uNK) cells are the predominant immune cell population at the maternal-fetal interface.^{31,32} Evidence from murine pregnancy also confirms the abundance of uNK cells during early to midpregnancy.^{30,33,34} The precise function of uNK cells at the maternal-fetal function of uNK cells at the maternal-fetal interface are subject to debate. uNK cells have been thought to play an important physiologic role in pregnancy by regulating trophoblast invasion.^{35,36} Conversely, a novel pathogenic link has been elucidated between uNK cells and fetal resorption in IL-10^{–/–} mice.³⁰ These data, coupled with other studies,^{31,33–36} now offer a better understanding of the functional spectrum of uNK cells.

We propose that in addition to their developmental functions, uNK cells may serve as a fail-safe mechanism during pregnancy, effecting pregnancy termination in response to a robust inflammatory insult. Using IL-10^{–/–} mice to study lipopolysaccharide (LPS)-induced preterm parturition, we demonstrate that uNK cells play a key role in preterm labor and delivery and may exert cytotoxic functions through TNF α production and by migrating into the placenta. These findings suggest a novel mechanism of inflammation-induced preterm labor and delivery and may offer insights leading to therapeutic interventions.

MATERIALS AND METHODS

Mice

Mice were housed and mated in a specific pathogen-free facility under the care of our hospital's central research department. All mice (C57BL/6, C57BL/6 IL-10^{–/–} NOD, and NOD IL-10^{–/–}) were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice of 7–8

weeks' age were mated and each experimental group contained at least 3 mice. Syngeneic matings between C57BL/6 IL-10^{-/-} mice or allogeneic matings between C57BL/6 IL-10^{-/-} female mice (H-2^b) and NOD IL-10^{-/-} male mice (H-2^k), and congenic wild type matings were carried out. The day of vaginal plug appearance was designated gestational day (gd) 0. All protocols were approved by the Lifespan Animal Welfare Committee and carried out according to its guidelines.

In vivo treatment of pregnant mice

On gd 14, mice (wild type and IL-10^{-/-}) received intraperitoneal (IP) injections of *Escherichia coli* LPS (O26:B6) (Sigma Chemical, St. Louis, MO) initially at varying doses and subsequently at 0.5 µg/mouse in 100 µL of saline. NK cell depletion of mice was performed by IP injection of rabbit antiasialo GM1 (100 µL) Wako USA, Richmond, VA) or anti-NK1.1 (PK-136) (250 µg) (BD Biosciences, San Jose, CA) on gd 11 and 14. Nonimmune rabbit serum (Antibodies Inc, Davis, CA) 100 µL) or irrelevant Isotype antibody was injected in parallel as controls. Anti-interferon (IFN)-γ (XMG1.2) and anti-TNFα (G281-2626) monoclonal antibodies (BD Biosciences) were administered IP at 750 and 300 µg, respectively, on gd 13 and 14. Recombinant IL-10 (R&D Systems, Minneapolis, MN) was administered prior to LPS on gd 14 at a dose of 500 ng/mouse.

Cellular isolation

Uterine mononuclear cells (UMC) were obtained by mechanical dispersion of gd 16 uteroplacental tissue in Roswell Park Memorial Institute 1640 supplemented with 10% fetal bovine serum, penicillin/streptomycin, and L-glutamine. Mononuclear cells were then isolated via density gradient separation using Fico-Lite LM (Atlanta Biologicals, Atlanta, GA).

Flow cytometry

UMC were stained with anti-CD45 (30-511), anti-NK1.1 (PK136), and anti-CD3 (145-2C11) (BD Biosciences). Intracellular cytokine staining for TNFα and IFN-γ was performed using a Cytofix/ Cytoperm kit according to the manufacturer's protocol (BD Biosciences) and anti-IFN-γ (XMG1.2) or anti-TNFα (G281-2626) monoclonal antibodies (BD Biosciences).

NK cell cytotoxicity assay

uNK cell cytotoxicity was measured using a flow cytometry-based assay according to the manufacturer's protocol (Molecular Probes, Eugene, OR). Purified anti-TNFα (G281-2626) monoclonal antibody (BD Biosciences) was added to the cultures of YAC-1 cells as indicated. Recombinant mouse TNFα (R&D Systems) was added to cultures of YAC-1 cells at doses of 0.01, 0.05, 0.1, 0.5, and 1 ng/mL in the absence of effector UMC.

Histochemistry

Individual uteroplacental units were isolated at gd 16 and fixed with 10% buffered formalin for 24 hours. The tissue was then paraffin-embedded and prepared for histologic staining with haematoxylin and periodic acid-Schiff reagent as previously described.³⁴ Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed using a kit according to the manufacturer's protocol (Chemicon, Temecula, CA).

Progesterone (P4) enzyme immunoassay

Serum samples from wild type of IL-10^{-/-} mice were collected on gd 16 and P4 levels were measured by a specific enzyme immunoassay kit (ALPCO Diagnostics, Salem, NH)

according to the manufacturer's protocol. These results were also confirmed with a different P4 enzyme immunoassay kit (Assay Desings Inc, Ann Arbor, MI).

Statistical Analysis

Statistical significance of pregnancy outcomes was examined using 1-way analysis of variance method. The Student *t* test was also used if required. Data are expressed as means, with differences represented by $\pm$ SD. A *P* value $\leq .05$ was considered to be statistically significant.

RESULTS

Low-dose LPS administration induces preterm labor and delivery in IL-10^{-/-} mice

We and others have shown that C57BL/6 IL-10^{-/-} mice experience full-term pregnancy.^{30,37} We initially found that doses > 0.5 μ g/mouse of LPS administered IP on gd 14 resulted in severe uteroplacental pathology (fetal death with necrosis) in C57BL/6 IL-10^{-/-} mice when examined on day 16 of pregnancy. Accordingly, we chose a dose of 0.5 μ g/mouse LPS to study its effects on the induction of preterm labor and delivery. We observed that this dose did not affect pregnancy in wild type mice. Pregnant C57BL/6 IL-10^{-/-} or wild type females were injected IP with 0.5 μ g LPS in 100 μ L of saline or saline vehicle on gd 14. LPS administration resulted in spontaneous preterm labor and delivery of live pups around gd 17 in IL-10^{-/-} in both syngeneic and allogeneic matings, but not in wild type mice (Table 1).

LPS treatment late in gestation induces increased uNK cell numbers in cytotoxic activation in IL-10^{-/-} mice

Although uNK cells are much reduced during later stages of pregnancy, we speculated that inflammation influenced the uNK cell number and function late in gestation, particularly in the absence of IL-10. In wild type animals, uNK cell numbers, as assessed by the proportion of CD45⁺NK1.1⁺CD3⁻ UMC, peaked around gd 12–13 (~20–25%) but the numbers were greatly reduced by gd 16 (~8–12%), in agreement with the published uNK cell population kinetics during pregnancy.^{31,33,34} We thus examined the effects of low-dose LPS treatment on uNK cells late in gestation in wild type or IL-10^{-/-} syngeneic matings. Mice were treated as previously described on gd 14, and on gd 16 UMC were isolated and the proportion of uNK cells was assessed by flow cytometry. In response to LPS, the proportion of uNK cells (NK1.1⁺CD3⁻) among total CD45⁺ population of UMC increased significantly in IL-10^{-/-} but not in wild type matings (Figure 1, A). These data indicate that low-dose LPS preferentially affect the uNK cell proportion in IL-10^{-/-} mice. This is significant given the normal decline in uNK cell numbers observed by gd 16.

To investigate the effects of LPS on uNK cell activation, UMC were isolated on gd 16 from pregnant IL-10^{-/-} and wild type mice and their cytotoxicity against the NK cell-sensitive YAC-1 cell line was assessed. LPS treatment in IL-10^{-/-} mice markedly increased uNK cell cytotoxicity as compared with LPS-treated wild type mice (Figure 1, B). Consistent with the lack of an effect of LPS treatment on splenic NK cell amplification, the cytotoxicity of these cells was unaffected in wild type and IL-10^{-/-} mice (data not shown). Thus, low-dose LPS administration in IL-10^{-/-} mice results in NK cell cytotoxic activation specific to the uterine compartment.

LPS induces migration of uNK cells into the placental zone in IL-10^{-/-} mice

uNK cells are localized to the mesometrial decidua during normal pregnancy.^{30,31,33,34} Given the observed late pregnancy LPS-induced enhancement of uNK cell number and cytotoxic activation in IL-10^{-/-} mice, we investigated the localization of uNK cells in these

animals. Using periodic acid-Schiff to visualize uNK cells and haematoxylin, we examined uteroplacental tissue isolated on gd 16 from syngeneically mated IL-10^{-/-} and wild type mice that had been treated with LPS or saline vehicle. In both IL-10^{-/-} (Figure 2, A) and wild type (Figure 2, B) mice treated with saline, a minor population of uNK cells remained in the mesometrial decidua. However, in LPS-treated IL-10^{-/-} mice, uNK cells infiltrated the placenta with a perivascular trafficking pattern (Figure 2, C). No such uNK cell infiltration was observed in LPS-treated wild type mice (Figure 2, D).

Depletion of uNK cells, neutralization of TNF α , or administration of IL-10 prevents preterm labor and delivery in LPS-treated IL-10^{-/-} mice

The increase in uNK cell number, cytotoxicity, and placental invasiveness suggests that these cells play a prominent role in the pathophysiology of LPS-induced preterm birth in IL-10^{-/-} mice. We sought to confirm the potential role of uNK cells in this process via NK cell depletion. Pregnant LPS-treated IL-10^{-/-} mice were injected IP with anti-NK1.1, anti-asialo GM1 antibodies, or control IgG on gd 8, 11, and 14, and LPS on gd 14. On gd 16, UMC were isolated and uteroplacental tissue was fixed for histology. Successful depletion of uNK cells was confirmed by flow cytometry (Figure 3, A). Histologic evaluation also confirmed the depletion of uNK cells (data not shown). Anti-asialo GM1 or NK1.1 antibody treatment abrogated the LPS-induced increase in uNK cell cytotoxicity, consistent with successful uNK cell depletion (Figure 3, B).

We next assessed the effect of uNK cell depletion, treatment with recombinant mouse IL-10, or neutralizing antibodies to TNF α or IFN- γ on preterm labor and delivery in LPS-treated IL-10^{-/-} mice. We demonstrated that NK cell depletion restored term delivery (Table 2). IL-10, as expected, also restored term delivery. Importantly, neutralization of TNF α had a much more profound effect in preventing preterm delivery than that observed by the NK-cell derived cytokine IFN- γ (Table 2).

TNF α is a key regulator of uNK cell invasion and cytotoxic activation

Given that uNK cell depletion and TNF α neutralization both prevented preterm deliveries in LPS-treated IL-10^{-/-} mice, we investigated relationships between uNK cells and TNF α . There was a marked increase in TNF α , and, to a lesser degree, IFN- γ -producing uNK cells in LPS-treated IL-10^{-/-} mice (Figure 4, A). Perforin/granzyme granule exocytosis is a major cytotoxic pathway of NK cells, whereas evidence for TNF α mediated NK cell cytotoxicity is sparse. Given the LPS-induced production of TNF α in uNK cells from IL-10^{-/-} mice, we examined the role of TNF α in LPS-mediated uNK cell cytotoxicity. UMC from LPS-treated IL-10^{-/-} mice were isolated and NK cell cytotoxicity was assessed after NK cell cytotoxicity was assessed after TNF α neutralization. uNK cell cytotoxicity was significantly reduced after TNF α neutralization (Figure 4, B), suggesting that uNK cell-derived TNF α may be a major mediator of uNK cell cytotoxicity in the IL-10^{-/-} mouse model. To further confirm that TNF α was directly cytotoxic in our assay, we cultured YAC-1 cells alone in the presence of varying doses of TNF α . Indeed, TNF α exerted a dose-dependent cytotoxic effect on YAC-1 cells (Figure 4, C).

The infiltration of uNK cells into the placenta of LPS-treated IL-10^{-/-} mice suggests that uNK cells may exert a deleterious effect on placental cells, leading to premature parturition. Interestingly, in addition to triggering uNK cell infiltration, LPS induced placental cell apoptosis as detected by TUNEL staining (Figure 5, A). Importantly, neutralization of TNF α blocked uNK cell migration to the placenta (Figure 5, B). These findings demonstrate that TNF α plays a central role in the pathophysiology of LPS-induced preterm birth in IL-10^{-/-} mice. Moreover, our observations also suggest that uNK cell invasion into the placenta is a key mechanistic event.

LPS-induced preterm birth in IL-10^{-/-} mice is not associated with loss of P4

It has been reported that treatment of mice with large doses of LPS reduced plasma P4 prior to labor.³⁸ These results suggest an endocrine-mediated mechanisms of LPS-induced preterm birth. Although our findings support an immunologic mechanism for low-dose LPS-induced preterm birth in IL-10^{-/-} mice, we examined the potential endocrine contribution via measurement of serum progesterone levels (Figure 6). No changes were observed in serum P4 levels on gd 16 in LPS-treated pregnant IL-10^{-/-} mice and congenic wild type mice. Moreover, there was no change in serum P4 between LPS-treated pregnant IL-10^{-/-} mice experiencing preterm birth and their NK cell-depleted counterparts experiencing term pregnancy, strongly implicating an uNK cell-mediated mechanism for the observed preterm birth.

COMMENT

This study establishes that uNK cells play a central role in inflammation-induced preterm labor and delivery. IL-10 likely prevents preterm parturition by regulation uNK cell function in the presence of an inflammatory insult. Unlike wild type mice, very low doses of LPS can induce preterm birth in IL-10^{-/-} mice where uNK cells become cytotoxic, increase in number, and migrate to the placenta. Our findings suggest a maternal immune-mediated mechanism of LPS-induced premature birth with cytotoxic and migratory uNK cells invading fetal tissue resulting in pathology.

Evidence for an indispensable role for uNK cells in inflammation-induced preterm labor and delivery derives from NK cell depletion experiments in IL-10^{-/-} mice. This result is surprising given the population kinetics of uNK cells. In both mice and human beings, specialized uNK cells invade the uterus during decidualization, peak at midgestation, and decline until they are nearly absent at term.^{31,33} In LPS-treated IL-10^{-/-} mice, the uNK cell number was significantly higher than in wild type control groups during late gestation (Figure 1). Thus, it appears that LPS-induced inflammatory signals promote either proliferation of resident uNK cells or recruitment of uNK cell precursors to the uterus in IL-10^{-/-} mice. In vitro studies demonstrate LPS-mediated effects on human NK cells, including proliferation, cytotoxicity, and IFN- γ production.³⁹⁻⁴² Our data provide in vivo evidence for LPS-mediated uNK cell activation and show invasion of uNK cells into the placenta as a necessary step to induce preterm delivery. An alternative explanation for LPS-induced preterm delivery could be functional loss of progesterone (P4) as P4 is required for implantation and maintenance of pregnancy.⁴³ Because NK cell-depleted IL-10^{-/-} mice do not experience preterm birth in response to LPS and spectrum P4 levels are similar between LPS-treated IL-10^{-/-} and wild type mice (Figure 6), it appears that preterm labor and delivery in our model is independent of P4 activity. uNK cells appear to be a key cell population linking inflammatory insult to preterm labor and delivery.

The sensitivity of IL-10^{-/-} mice to LPS-induced uNK cell-associated preterm birth suggests that IL-10 plays a key regulatory role in uNK cell function and proliferation. Single dose administration of recombinant IL-10 restored pregnancy to term in these mice (Table 2). Invasion of uNK cells into the placenta of LPS-treated IL-10^{-/-} mice suggest aberrant production or regulation of chemotactic factors by the placenta. LPS can trigger the expression of various chemokines in macrophages and trophoblasts,^{44,45} whereas IL-10 acts to down-regulate these chemokines.^{46,47} The absence of this regulation in IL-10^{-/-} mice may contribute to abnormal cellular trafficking.

In vivo TNF α neutralization in LPS-treated IL-10^{-/-} mice prevents uNK cell invasion into the placenta, placental cell apoptosis, and preterm labor and delivery (Figure 5; and Table 2). TNF α induces the production of prostaglandins and metalloproteinases, molecules

associated with cellular infiltration.⁴⁸ During normal pregnancy, uNK cells remain noncytotoxic despite the presence of cytotoxic granules. We hypothesized that in LPS-treated IL-10^{-/-} mice, uNK cells may kill through perforin/granzyme granule-mediated mechanisms. Human uNK cells express molecules required for targeted cell lysis and are able to form conjugates and immune synapses with target cells, yet remain noncytotoxic.⁴⁹ Because TNF α was found to be a regulator of uNK cell cytotoxicity and migration (Figure 4 and 5), we conclude that the uNK cell-TNF α axis is an important mechanism for preterm labor and delivery in IL-10^{-/-} mice.

Our data suggest a regulatory role for IL-10 on uNK cells during an inflammatory insult leading to preterm parturition. It is plausible that pregnant women with premature reductions in the levels of IL-10 will experience preterm labor and delivery in response to even subclinical infection/inflammation. Thus, our results using IL-10^{-/-} mice provide a cogent framework for explanation of inflammation-associated preterm labor and delivery, leading to further studies in human beings and revealing potential targets for clinical interventions.

Acknowledgments

We thank members of the Sharma Laboratory for discussions and editorial assistance. P4 assays were performed by Satyan Kalkunte and Wendy Norris of our laboratory and thanks are due to them.

Supported in part by the Grants from National Institutes of Health National Center for Research Resources (P20RR018728), National Institutes of Environmental Health Sciences Superfund Basic Research Program Award (P42ES013660), and a Subcontract WSU05056 under NICHD Contract #N01-HD-2-3342. This research was also supported, in part, by the Intramural Research Program of the Eunice Kennedy Shriver NICHD, NIH, Department of Health and Human Services.

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Figure 1. Cytotoxic activation of uNK cells by LPS

Impact of lipopolysaccharide (LPS) on uterine (Ut) natural killer (NK) cell number and cytotoxic activation. LPS (0.5 μ g/mouse) or saline was injected intraperitoneally on gestational day (gd) 14 in wild type ($n=6$) or interleukin (IL)-10^{-/-} ($n=8$) mice. **A**, Ut mononuclear cells (UMC) were isolated on gd 16 from LPS- and saline-treated wild type and IL-10^{-/-} mice as described in “Materials and Methods” section. Proportions of Ut and splenic (Sp) NK cells were analyzed. Percentage of NK1.1⁺CD3⁻ cells is gated as part of CD45⁺ mononuclear cells and indicated in upper left quadrant of each graph. **B**, Isolated UMC on gd 16 were analyzed for NK cell cytotoxicity using YAC-1 target cells as described in “Materials and Methods” section. Uterine horns from 3 mice each of wild type and IL-10^{-/-} mice were processed for isolation of mononuclear cells for experiments in **A** and **B**.

E:T, effector: target cell ratio.

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Figure 2. LPS-induced migration of uNK cells

Uterine natural killer (uNK) cell localization in wild type and interleukin (IL)-10^{-/-} mice. Wild type and IL-10^{-/-} mice were injected intraperitoneally with saline or lipopolysaccharide (LPS) (0.5 µg/mouse) on gestation day (gd) 14 and killed on gd 16. Histologic sections of uteroplacental tissue were analyzed with periodic acid-Schiff-hematoxylin staining to visualize NK cells. In both saline-injected **B**, wild type and **A**, IL-10^{-/-} mice, uNK cells remain localized to mesometrial decidual regions (M1 and M2, *arrowheads*) and are not found within placenta (P). In contrast, LPS-injected **C**, IL-10^{-/-} mice exhibited profound infiltration of uNK cells into P zone (P1 and P2, *arrowheads*), which was not seen in P and **D**, wild type mice. Scale bar, 150 µm; insets, scale bar 15 µm. All analyses were carried out with tissues from at least 3 different experiments. Murphy. Evidence for participation of uterine natural killer cells in the mechanisms responsible for spontaneous preterm labor and delivery. Am J Obstet Gynecol 2009.

Figure 3. Depletion of uNK cells

Depletion of uterine natural killer (uNK) cells in lipopolysaccharide (LPS)-treated interleukin (IL)-10^{-/-} mice. **A**, IL-10^{-/-} mice were treated with either anti-asialo GM1 (AGM1) or anti-NK1.1 antibodies intraperitoneally on gestational day (Gd) 8, 11, and 14 or appropriate Isotype control antibodies. These mice then received 0.5 μ g LPS on gd 14. Uterine mononuclear cells (UMC) were isolated and analyzed by flow cytometry. Both anti-AGM1 and anti-NK1.1 treatments successfully depleted uNK cells. **B**, Consistent with successful uNK cell depletion, NK cell cytotoxicity was greatly diminished in UMC preparations from anti-AGM1 and anti-NK1.1 treated mice.

E:T, effector: target cell ratio.

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Figure 4. Impact of TNF α neutralization

Evidence for tumor necrosis factor (TNF)- α -mediated uterine natural killer (NK) cell cytotoxic activation in lipopolysaccharide (LPS)-treated interleukin (IL)-10 $^{-/-}$ mice. **A**, IL-10 $^{-/-}$ mice were treated with 0.5 μ g LPS or saline vehicle on gestational day (gd) 14 and uterine mononuclear cells (UMC) were isolated at gd 16. These cells were then stained for CD3, NK1.1, and either TNF α or interferon (IFN)- γ . Shown are events gated on NK1.1 $^{+}$ CD3 $^{-}$ population. In response to LPS, TNF α , and IFN- γ production was examined by intracellular staining and fluorescence-activated cell sorting analysis. **B**, IL-10 $^{-/-}$ mice were treated with 0.5 μ g LPS on gd 14 and UMC were then isolated at gd 16 and subjected to cytotoxicity assay using Yac-1 cell targets. **C**, Yac-1 cells were cultured in the presence of indicated concentrations of TNF α and analyzed for cell death.

E:T, effector: target cell ratio.

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Figure 5. TNF α production by uNK cells

Neutralization of tumor necrosis factor (TNF)- α blocks uterine natural killer (uNK) cell infiltration into placenta (P) and appearance of TUNEL-positive regions in interleukin (IL)-10 $^{-/-}$ mice. **A**, IL-10 $^{-/-}$ mice were treated with 0.5 μ g lipopolysaccharide (LPS) or saline vehicle on gestational day (gd) 14. Animals that received LPS also were treated with either anti-TNF α antibody or irrelevant IgG on gd 13 and 15. Mice were then killed and utero-P tissue was analyzed for uNK cell migration by periodic acid-Schiff (PAS) staining and cell death via TUNEL histology (without haematoxylin staining). uNK cells were not observed in P of mice receiving saline vehicle alone. Extensive P uNK cell (arrowhead) infiltration was observed in animals treated with LPS and control IgG. This uNK cell infiltration was associated with extensive P cell apoptosis as demonstrated by TUNEL-positive region (arrow). **B**, IL-10 $^{-/-}$ mice were treated with anti-TNF α antibody on gd 13 and 15 along with 0.5 μ g LPS on gd 14. Mice were then killed and utero-P tissue was analyzed for uNK cell migration by PAS/haematoxylin histology. Anti-TNF α antibody treatment prevented LPS-induced uNK cell invasion into P. uNK cells remained localized to mesometrial decidua (M) in these mice. Scale bar, 150 μ m; insets, scale bar 15 μ m. All analyses were carried out with tissues from at least 3 different experiments. Murphy. Evidence for participation of uterine natural killer cells in the mechanisms responsible for spontaneous preterm labor and delivery. Am J Obstet Gynecol 2009.

Figure 6. Serum progesterone levels in mice

Lipopolysaccharide (LPS)-induced preterm birth is not due to loss of P4 levels in treated mice. Circulating levels of P4 were estimated on gestational day 16 in wild type (WT) and interleukin (IL)-10^{-/-} mice ($n=3$) treated with either saline or LPS with or without anti-asialo GM1 (AGM1) or anti-natural killer (NK)1.1. No treatment groups showed any statistically significant change in levels of P4 as compared with saline treated controls. Murphy. Evidence for participation of uterine natural killer cells in the mechanisms responsible for spontaneous preterm labor and delivery. *Am J Obstet Gynecol* 2009.

Table 1

Impact of lipopolysaccharide on gestational length.

Mating combination	Treatment	Mean day of birth (gd)	<i>P</i> value
Syngeneic matings			
C57BL/6 IL-10 ^{-/-} × C57BL/6 IL-10 ^{-/-}	Saline (<i>n</i> = 9)	19.6 ± 1.01	< .0001
C57BL/6 IL-10 ^{-/-} × C57BL/6 IL-10 ^{-/-}	LPS (<i>n</i> = 17)	16.5 ± 0.73	
C57BL/6 × C57BL/6	Saline (<i>n</i> = 9)	19.8 ± 0.67	.7439
C57BL/6 × C57BL/6	LPS (<i>n</i> = 22)	19.7 ± 0.80	
Allogeneic matings			
C57BL/6 IL-10 ^{-/-} × NOD IL-10 ^{-/-}	Saline (<i>n</i> = 3)	19.3 ± 0.58	0.261
C57BL/6 IL-10 ^{-/-} × NOD IL-10 ^{-/-}	LPS (<i>n</i> = 3)	17.0 ± 1.00	
C57BL/6 × NOD	Saline (<i>n</i> = 6)	19.6 ± 0.55	0.774
C57BL/6 × NOD	LPS (<i>n</i> = 5)	19.5 ± 0.52	

gd, gestational day; *IL*., interleukin; *LPS*, lipopolysaccharide.

All mice received saline or LPS treatment (0.5 µg/mouse) on gd 14. Gestational length was determined according to day of delivery. Data represent multiple matings as representative by *n* values. Statistical significance of pregnancy duration was examined using Student *t* test. Data are expressed as means ± SD. A *P* value .05 was considered to be statistically significant.

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Table 2

Rescue of lipopolysaccharide-induced preterm birth in interleukin-10^{-/-} mice.

Treatment	Mean day of birth (gd)	P value
LPS + IgG (n = 6)	17.2 ± 0.75	
LPS + NRS (n = 5)	17.0 ± 1.00	.7130
LPS + anti-AGM1 (n= 12)	19.3 ± 0.98	.0003
LPS + anti-NK1.1 (n= 4)	19.5 ± 0.58	.0009
LPS + anti-TNF α (n= 5)	19.6 ± 0.55	.0002
LPS + anti-IFN- γ (n= 6)	18.5 ± 1.25	.0538
LPS + mrIL-10 (n = 4)	19.3 ± 0.96	.0160

AGM1, asialo GM1; *gd*, gestational day; *IFN*, interferon; *mrIL*, mouse recombinant interleukin; *LPS*, lipopolysaccharide; *NK*, natural killer; *NRS*, nonimmune rabbit serum; *TNF*, tumor necrosis factor.

For NK cell depletion, mice were administered with anti-AGM1, anti-NK1.1, or corresponding control antibodies on gd 11 and 14. For anti-IFN- γ or anti-TNF α antibody treatment, mice were administered with antibodies on gd 13 and 14. All LPS injections were performed on gd 14. mrIL-10 was injected intraperitoneally 15 minutes prior to LPS administration on gd 14. *P* values are compared with pregnancy outcome in mice receiving LPS + IgG.

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