Published in final edited form as:

Reprod Sci. 2008 February ; 15(2): 121–127. doi:10.1177/1933719107310992.

Pre-treatment with Toll-like receptor 4 antagonist inhibits lipopolysaccharide-induced preterm uterine contractility, cytokines, and prostaglandins in rhesus monkeys

Kristina M. Adams Waldorf^{1,2}, David Persing³, Miles J. Novy², Drew W. Sadowsky², and Michael G. Gravett^{1,2}

¹University of Washington, Seattle, WA

²Oregon National Primate Research Center, Beaverton, OR

³Cepheid, Inc., Sunnyvale, CA

⁴GlaxoSmithKline, Inc., Philadelphia, PA

Abstract

Intra-uterine infection, which occurs in the majority of early preterm births, triggers an immune response culminating in preterm labor. We hypothesized that blockade of lipopolysaccharide (LPS)-induced immune responses by a Toll-like receptor 4 antagonist (TLR4A) would prevent elevations in amniotic fluid (AF) cytokines, prostaglandins, and uterine contractility. Chronically catheterized rhesus monkeys at 128–147 days gestation received intra-amniotic infusions of either: 1) saline (n=6), 2) LPS (0.15–10 µg; n=4), or 3) TLR4A pre-treatment with LPS (10 µg) one hour later (n=4). AF cytokines, prostaglandins, and uterine contractility were compared using oneway ANOVA with Bonferroni-adjusted pairwise comparisons. Compared to saline controls, LPS induced significant elevations in AF IL-8, TNF- α , PGE₂, PGF₂ α , and uterine contractility (p<0.05). In contrast, TLR4A pre-treatment inhibited LPS-induced uterine activity and was associated with significantly lower AF IL-8, TNF- α , PGE₂, and PGF₂ α versus LPS alone (p<0.05). Toll-like receptor antagonists, together with antibiotics, may delay or prevent infection-associated preterm birth.

Keywords

TLR4; intrauterine infection/inflammation; preterm labor; rhesus monkey; animal model

INTRODUCTION

Intra-amniotic infection (IAI), present in most cases of early preterm birth, triggers an immune response thought to result in preterm labor.^{1, 2} Elevations in amniotic fluid pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6, IL-8) and prostaglandins (PGE₂, PGF₂ α) have been reported in women and rhesus monkeys with intra-amniotic infection and preterm labor suggesting that pro-inflammatory mediators play a key role in triggering uterine contractions.^{3–6} We previously demonstrated that immunomodulators (indomethacin, dexamethasone, and interleukin-10) significantly inhibited uterine activity and the pro-inflammatory cascade induced by intra-amniotic infusion of interleukin-1 β (IL-1 β) in a non-human primate (NHP) model of preterm labor.^{7, 8} Furthermore, dexamethasone and indomethacin administered with

antibiotics prolonged gestation and suppressed amniotic fluid cytokine and prostaglandin production in response to experimental IAI with GBS.⁹ These immunomodulators targeted cytokine and prostaglandin production, which likely represent downstream events in the inflammatory cascade and may not protect the fetus from adverse sequelae of intrauterine infection. Further inhibition of uterine activity and the fetal inflammatory response could result from blockade of the initial events in immune recognition of bacteria mediated by toll-like receptors (TLR), which are the principal and earliest sensors of bacterial pathogens.¹⁰

To determine whether blockade of TLR would further inhibit preterm labor and the cytokine-prostaglandin cascade, we developed a lipopolysaccharide (LPS) model of preterm birth. LPS is the major component of the outer membrane of gram-negative bacteria and is an inflammatory stimulus not associated with bacterial tissue invasion that could introduce confounding experimental variables. Toll-like receptor 4 (TLR4) recognizes LPS and triggers pro-inflammatory cytokine gene expression.¹¹⁻¹³ TLR4 is abundantly expressed in the placenta including the amniotic epithelium and increased chorioamniotic expression has been described with intrauterine infection.¹⁴⁻¹⁶ Although intrauterine injection of LPS induces preterm birth in many murine models, administration of LPS to TLR4 mutant mice (C3H/HEJ) does not result in preterm delivery.¹² Experimental dissection of the immune response is difficult when using a live pathogen due to confounding variables introduced by microbial invasion or tissue damage. Antagonism of TLR4 in an LPS model allows investigation of the specific effect of blocking the earliest immune responses to intra-amniotic bacterial products without confounding variables introduced by a live pathogen such as microbial invasion and tissue damage.

Our study hypothesis was that intra-amniotic infusion of a TLR4A inhibits LPS-induced uterine contractility and the cytokine-prostaglandin cascade in a nonhuman primate model. To investigate this hypothesis, we used a long-term catheterized model in pregnant rhesus monkeys in which placentation and the endocrine and paracrine control of parturition is similar to human pregnancy. We infused LPS into the amniotic cavity of stable catheterized non-human primates and studied the effect of TLR4 antagonism on uterine activity and on the endogenous production of cytokines, chemokines, prostaglandins, and matrix metalloproteinase-9 (MMP-9). To our knowledge this study is the first to demonstrate the immunologic and physiologic consequences of an intraamniotic LPS stimulus in a NHP model and to test “proof of concept” in applying TLR4 antagonism as a novel interventional strategy.

MATERIAL AND METHODS

Animals and Study Groups

Study protocols were approved by the Institutional Animal Care and Utilization Committee and guidelines for humane care were followed. Timed-pregnant rhesus monkeys (*Macaca mulatta*) were adapted to a vest and mobile catheter protection device as previously described.¹⁷ Intrauterine surgery was performed on average at 123 days gestation (range 119-126) to implant fetal ECG electrodes and catheters in the amniotic fluid, maternal femoral vein and artery, fetal jugular vein and fetal carotid artery. Postoperative intravenous infusions included 250 mg cefazolin sodium q12 hours for 5 days to prevent infection and either terbutaline sulfate (Bricanyl, Merrel Dow Pharm. Inc., Kansas City, MO) or atosiban (Merck & Co., Inc., West Point, PA) for 1-5 days to control uterine irritability. At our center, term gestation in the non-instrumented rhesus monkey population averages 167 days (range 155-172 days).

Experimental intra-amniotic infection was simulated by intra-amniotic inoculation of LPS in eight animals. Four of these animals were observed without treatment after receiving 150 ng (n=1), 1 µg (n=1), or 10 µg (n=2). The remaining four animals received intra-amniotic inoculations of 10 mg TLR4 antagonist followed by 10 µg of LPS one hour later, which

represents a 1,000-fold excess of antagonist to agonist. To determine whether TLR4 antagonism of LPS induction of intrauterine inflammation was effective one week after initial inoculation, we administered 10 mg LPS into the amniotic fluid. An additional six animals received only saline intra-amniotic inoculations serving as controls and have been previously published.

LPS and TLR4A

The LPS was from *Escherichia coli* (L3024, O111:B4 serotype, Sigma Chemical Co., St. Louis, MO) and purified by phenol extraction and ion exchange chromatography. LPS was then reconstituted in endotoxin-free water (210-7, Sigma). The lot used was estimated to have a protein contamination level of 0.3% and 1,250,000 endotoxin units per mg. The TLR4A is a synthetic lipid A analog, the toxic portion of LPS (GlaxoSmithKline Biologicals, Research Triangle Park, NC).¹⁸ The TLR4A was provided as a gift from GlaxoSmithKline.

Uterine Activity, Preterm Labor, Cesarean Section

Intraamniotic pressure was continuously recorded from the time of surgery, digitized, and analyzed as previously described.⁶ The integrated area under the intrauterine pressure curve was used as the measure of uterine activity and reported as the hourly contraction area (HCA; mmHg•sec/hr) over 24 hours. Preterm labor was defined as >10,000 mmHg•sec/hr associated with a change in cervical effacement or dilation. Cesarean section was performed in order to optimize the collection of intact gestational tissues when vaginal delivery was considered to be imminent. After cesarean section, fetuses were euthanized by barbiturate overdose followed by exsanguination and fetal necropsy. Complete gross and histopathologic examination was performed on infants and placentas.

Quantitation of Amniotic Fluid Cytokines, Prostaglandins, and Matrix Metalloproteinases

Beginning 48 hours prior to bacterial inoculation, amniotic fluid was sampled daily until delivery. Samples were centrifuged and the supernatant frozen and stored at -20°C. Prior to freezing, ethylenediaminetetraacetic acid (7.9 mM) and indomethacin (0.3 mM) was added to samples saved for prostaglandin quantitation to prevent prostaglandin metabolism. Quantities of IL-1 β , IL-6, IL-8, PGE₂, and PGF₂ α were determined using commercially available human ELISA (BioSource International, Camarillo, CA) and EIA kits (Cayman Chemical, Ann Arbor, MI). TNF- α concentrations were determined by rhesus monkey-specific ELISA (BioSource International, Camarillo, CA). Standard gelatin zymography was used to semi-quantitate the activity of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) in amniotic fluid, adapted from previously published methods. In brief, all amniotic fluid samples (5 μ l volume) from one animal were loaded onto a gelatin-containing polyacrylamide gel, electrophoresed at constant voltage, digested for 24h, and stained with Coomassie blue R-250. Gels were scanned and densitometric analysis of integrated area of lysis was conducted using Scion Image (NIH). Comparisons of MMP-9 concentrations were conducted on percent change from average baseline to average post-inoculation sample for each animal.

Statistical Analysis

Study outcomes were quantities of uterine activity (mean 24-hour HCA), amniotic fluid cytokines (IL-1 β , TNF- α , IL-6), prostaglandins (PGE₂, PGF₂ α), and matrix metalloproteinases (MMP-2, MMP-9) during peak response in the first 7 days after LPS inoculation and are presented as mean and standard error of the mean. Data was transformed by natural logarithm prior to analysis with the exception of matrix metalloproteinase data. Prior to log transformation of IL-1 β and IL-6, zero values were recoded as half of the lowest value detected by the ELISA assay (0.2 and 4.0 pg/ml, respectively). All statistical analyses were conducted using Intercooled STATA 8.2 for Windows 2000 (StatCorp, College Station, TX) compared using

oneway analysis of variance with pairwise comparisons adjusted using Bonferroni. Significance was accepted at $p < 0.05$.

RESULTS

Uterine Activity

The mean gestational age of inoculation was 135 days (range 128-137). A representative animal from each experimental group is shown in Figure 1. Prior to LPS inoculation, the uterus was quiescent in all animals with an average HCA less than 1,250 mmHg•sec/hr (5,000-10,000 HCA: moderate uterine activity; >10,000 HCA: preterm labor). LPS inoculation was associated with a significant increase in uterine contractility compared to saline infusion alone (Figure 2A: LPS: 4593 ± 3321 HCA, Saline 267 ± 91 HCA; $p = 0.02$). Increases in uterine contractility occurred 4-15 hours after LPS inoculation with contractions generally building with peak uterine activity achieved within 4-6 days. In one animal receiving 150 ng intra-amniotic LPS, uterine activity patterns met our center's criteria for delivery resulting in cesarean section on day 6 post inoculation. In contrast, pre-treatment with a TLR4A largely ablated this increase and uterine contractility did not differ significantly from saline controls (Figure 2A: LPS + TLR4A 1304 ± 372 HCA). In the animals treated with a TLR4A, a repeat LPS challenge one week later was associated with only modest increases in uterine activity, (2728 ± 1398 HCA). In a single animal, the second LPS challenge was associated with preterm labor and cesarean section was performed 24 hours post-inoculation.

Cytokines and Prostaglandins

Following LPS intra-amniotic infusion, predictable increases in amniotic fluid cytokines and prostaglandins were observed (Figures 1, 2B, 2C) followed by increased uterine contractility. LPS infusion alone was associated with significant increases in TNF- α ($p < 0.001$) and IL-8 ($p = 0.02$). In contrast, pre-treatment with a TLR4 antagonist prevented increases in cytokines, prostaglandins, and uterine contractility. Pre-treatment with a TLR4A resulted in decreases in IL-1 β and significant reductions in TNF- α ($p < 0.01$), and IL-8 ($p = 0.02$) compared to LPS infusion alone. Similarly LPS infusion resulted in significant increases in PGE₂ ($p = 0.02$) and PGF_{2 α} ($p = 0.01$) versus saline controls that were reduced by TLR4A pre-treatment (PGE₂: $p = 0.002$; PGF_{2 α} : $p = 0.05$). Amniotic fluid leukocytes followed a similar pattern to that of cytokines and prostaglandins and were significantly increased with LPS infusion alone versus saline controls ($p = 0.001$) and reduced by TLR4A pre-treatment ($p < 0.05$; data not shown).

Matrix Metalloproteinases

To study the effect of LPS infusion and TLR4 antagonism on MMP, we measured the activity of MMP-2 and MMP-9 semi-quantitatively by zymography and quantitated protein by Western blot in the LPS and TLR4A groups only. Neither LPS infusion nor TLR4A pre-treatment was associated with a significant change in either the active or pro-enzyme forms of MMP-2 or MMP-9. There were also no significant differences between LPS and TLR4A groups in either the active or pro-enzyme forms of MMP-2 or MMP-9 (data not shown).

COMMENT

Our study objective was to determine whether blockade of the earliest (innate) immune response to a bacterial pathogen would inhibit preterm labor, cytokines, and prostaglandins. Our experimental system took advantage of a novel immunomodulator, a TLR4A, which inhibits LPS signaling that occurs during gram-negative bacterial infections. Creation of an LPS model of preterm birth in the NHP was a necessary first step in testing our hypothesis and had not previously been established. Prior animal models (i.e. murine, rabbit) induced preterm labor by LPS injection into the peritoneal cavity, gestational sacs, or amniotic fluid.¹⁹⁻²¹ Data

from these animal models could not be used in predicting LPS sensitivity in the NHP amniotic cavity, because lower mammals are significantly less sensitive to LPS than humans.²² As anticipated, the dose of LPS resulting in preterm labor in NHP was several orders of magnitude less than required for the mouse model. One study limitation was the use of LPS doses lower than 10 µg in the LPS infusion group, which acted to bias against our finding of a significant difference between the LPS and TLR4A pre-treatment groups. The use of lower LPS doses occurred in earliest phase of the experiments and was done to reduce the possibility of a maternal or fetal death due to insufficient knowledge regarding the effect of different doses of intra-amniotic LPS in NHP and humans. Despite use of even very low doses, LPS infusion induced significant increases in uterine activity, amniotic fluid TNF-α, IL-8, PGE₂, PGF₂α, and leukocytes. The temporal pattern of cytokine and prostaglandin upregulation after LPS infusion was similar to observations during intraamniotic infection with Group B *streptococcus* and *Ureaplasma parvum*.^{6, 23} This is not surprising as the macrophage activation program of gene responses to whole bacteria is highly congruent to that observed with LPS alone.²⁴

In contrast, pre-treatment with a TLR4A prior to LPS infusion was associated with no significant increases in uterine activity, cytokines, or prostaglandins versus saline controls. Pre-treatment with the TLR4A was also associated with significant reductions in amniotic fluid TNF-α, IL-8, prostaglandins, and leukocytes compared to LPS infusion alone. Surprisingly, a repeat LPS challenge one week after TLR4A pre-treatment resulted in only modest increases in uterine activity in 3 of 4 animals, suggesting a sustained effect of the drug over this time period. Although one might predict *a priori* that antagonist pre-treatment would ablate the effect of an agonist, this assumption must be tested empirically in the course of drug discovery. Pre-treatment with a TLR4 antagonist might not have been effective *in vivo*, due to rapid drug clearance from the amniotic fluid or inability to completely saturate LPS docking sites within the fetal membranes.

Our preliminary data revealed no significant side effects of the TLR4A in our NHP model, such as fever or complement activation, which could potentially occur in an otherwise nontoxic lipid A analog (data not shown).²⁵ No significant adverse events have been observed with the use of other TLR4A in animals and humans *in vivo*. Neither of these properties has been observed during *in vivo* studies of E5564, another synthetic lipid A analog (Eisai, Inc., Woodcliff Lake, NJ) or with this TLR4A, but will be thoroughly investigated in future studies.^{26, 27} Secondly, although the drug half-life and clearance of this TLR4A is unknown, it is likely similar to lipid A and E5564. Lipid A and E5564 form complexes with high-density lipoproteins (HDL) in plasma and serum rapidly after administration, which reduces toxicity of lipid A and the TLR4A activity of E5564.²⁸ Amniotic fluid HDL levels are approximately 200-fold lower than in peripheral blood, therefore, the half-life of a synthetic lipid A analog should be significantly longer in the amniotic cavity. In human pharmacokinetic studies of E5564, TLR4A activity was measurable up to 8 hours after a 30-minute infusion, but declined more rapidly in lower doses and with bolus dosing.²⁹ The combination of bolus dosing and low amniotic fluid HDL levels may have contributed to the observed sustained TLR4A activity even one week after initial dosing. Additional studies of graded dose-response relationships, pharmacokinetics and placental transfer, including intraamniotic mechanisms which selectively sequester or degrade TLR4A, and potential fetal side effects remain to be determined.

We consider this work as proof of concept that blockade of TLR, the innate immune response to bacterial pathogens, is effective in blocking cell signaling culminating in uterine contractions and the cytokine-prostaglandin cascade. Our prior studies have already demonstrated that other generalized immunomodulators targeting later events in the immune response, such as cytokine and prostaglandin synthesis, are also effective in inhibiting contractions in our NHP model.

Dexamethasone (corticosteroid; cytokine suppressant), indomethacin (inhibitor of prostaglandin synthesis), and interleukin-10 (IL-10; anti-inflammatory cytokine) have been studied in our NHP model.^{7, 8} All three drugs inhibited cytokine-induced (IL-1 β) uterine contractility, but differed in their ability to suppress specific amniotic fluid cytokines and prostaglandins. Although intra-amniotic and intravenous IL-10 inhibited TNF- α and prostaglandins, it was impractical to administer and did not suppress other cytokines and hormones. We have recently demonstrated that ampicillin plus dexamethasone and indomethacin delayed preterm birth induced by Group B Streptococcus by 8 days.⁹ It is possible that TLR blockade of the innate immune response, in combination with antibiotics, will result in a longer latency period or perhaps even prevention of infection-induced preterm birth.

There is a prevalent view that early diagnosis and treatment of intra-amniotic infection is not reliable or cost effective and subsequent preterm labor is irreversible. We disagree with this nihilistic view for two reasons; infection-induced preterm labor in the NHP model is delayed by anti-inflammatory drugs and recent studies suggest that rapid identification of women with intra-amniotic infection will become possible. After experimental infection in the NHP model, novel amniotic fluid peptides were discovered by proteomics-based analysis as early as 12 hours after infection.³⁰ Two of the four amniotic fluid peptides, calgranulin and a unique fragment of insulin-like growth factor binding protein 1 (IGF-BP1), were also identified in the amniotic fluid of women with subclinical intra-amniotic infection and preterm labor. Detection of calgranulin and the IGF-BP1 peptide was even possible in maternal serum. A rapid screening test for these peptides would allow obstetricians to diagnosis intra-amniotic infection at an early stage, when treatment is likely to be successful. Future therapeutic trials would also be able to target a well-defined group of women with intrauterine infection.

In summary, TLR4 blockade may become a critical component in treating preterm labor induced by gram-negative bacteria. The combination of blocking TLR, the earliest step in the immune response, antibiotic treatment of the infection, and downregulation of cytokine and prostaglandin synthesis is the cornerstone of our conceptual model in investigating novel interventional or treatment strategies for infection-induced preterm labor.

Acknowledgments

The authors gratefully acknowledge the expert technical assistance of Michael Cook, Noreen Currier, Allison Watts, and Jan Hamanishi.

Supported by NIH grants HD01264, AI067910, AI42490, HD06159, P51-RR000163

REFERENCES

1. Chellam VG, Rushton DI. Chorioamnionitis and funiculitis in the placentas of 200 births weighing less than 2.5 kg. *Br J Obstet Gynaecol* 1985;92:808–814. [PubMed: 4027203]
2. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. *N Engl J Med* 1988;319:972–8. [PubMed: 3262199]
3. Romero R, Manogue KR, Mitchell MD, et al. Infection and labor. IV. Cachectin-tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor. *Am J Obstet Gynecol* 1989;161:336–41. [PubMed: 2764054]
4. El-Bastawissi AY, Williams MA, Riley DE, Hitti J, Krieger JN. Amniotic fluid interleukin-6 and preterm delivery: a review. *Obstet Gynecol* 2000;95:1056–64. [PubMed: 10808034]
5. Romero R, Ceska M, Avila C, Mazor M, Behnke E, Lindley I. Neutrophil attractant/activating peptide-1/interleukin-8 in term and preterm parturition. *Am J Obstet Gynecol* 1991;165:813–20. [PubMed: 1951537]

6. Gravett MG, Witkin SS, Haluska GJ, Edwards JL, Cook MJ, Novy MJ. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *Am J Obstet Gynecol* 1994;171:1660–7. [PubMed: 7802084]
7. Sadowsky DW, Haluska GJ, Gravett MG, Witkin SS, Novy MJ. Indomethacin blocks interleukin 1beta-induced myometrial contractions in pregnant rhesus monkeys. *Am J Obstet Gynecol* 2000;183:173–80. [PubMed: 10920327]
8. Sadowsky DW, Novy MJ, Witkin SS, Gravett MG. Dexamethasone or interleukin-10 blocks interleukin-1beta-induced uterine contractions in pregnant rhesus monkeys. *Am J Obstet Gynecol* 2003;188:252–63. [PubMed: 12548226]
9. Gravett MG, Adams KM, Sadowsky DW, et al. Immunomodulators plus antibiotics delay preterm delivery after experimental intra-amniotic infection in a nonhuman primate model. *Am J Obstet Gynecol*. 2007in press
10. Miyake K. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. *Semin Immunol* 2007;19:3–10. [PubMed: 17275324]
11. Medzhitov R, Preston-Hurlburt P, Janeway CA JR. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 1997;388:394–7. [PubMed: 9237759]
12. Poltorak A, He X, Smirnova I, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 1998;282:2085–8. [PubMed: 9851930]
13. Poltorak A, Smirnova I, He X, et al. Genetic and physical mapping of the *Lps* locus: identification of the toll-4 receptor as a candidate gene in the critical region. *Blood Cells Mol Dis* 1998;24:340–55. [PubMed: 10087992]
14. Adams KM, Lucas J, Kapur RP, Stevens AM. LPS induces translocation of TLR4 in amniotic epithelium. *Placenta* 2007;28:477–81. [PubMed: 17055575]
15. Abrahams VM, Mor G. Toll-like receptors and their role in the trophoblast. *Placenta* 2005;26:540–7. [PubMed: 15993703]
16. Kim YM, Romero R, Chaiworapongsa T, et al. Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. *Am J Obstet Gynecol* 2004;191:1346–55. [PubMed: 15507964]
17. Ducusy CA, Cook MJ, Novy MJ. Simplified vest and tether system for maintenance of chronically catheterized pregnant rhesus monkeys. *Lab Anim Sci* 1988;38:343–4. [PubMed: 3411932]
18. Fort MM, Mozaffarian A, Stover AG, et al. A synthetic TLR4 antagonist has anti-inflammatory effects in two murine models of inflammatory bowel disease. *J Immunol* 2005;174:6416–23. [PubMed: 15879143]
19. Elovitz MA, Wang Z, Chien EK, Rychlik DF, Phillippe M. A new model for inflammation-induced preterm birth: the role of platelet-activating factor and Toll-like receptor-4. *Am J Pathol* 2003;163:2103–11. [PubMed: 14578208]
20. Hirsch E, Saotome I, Hirsh D. A model of intrauterine infection and preterm delivery in mice. *Am J Obstet Gynecol* 1995;172:1598–603. [PubMed: 7538729]
21. McDuffie RS JR, Sherman MP, Gibbs RS. Amniotic fluid tumor necrosis factor-alpha and interleukin-1 in a rabbit model of bacterially induced preterm pregnancy loss. *Am J Obstet Gynecol* 1992;167:1583–8. [PubMed: 1471670]
22. Berczi I, Bertok L, Bereznoi T. Comparative studies on the toxicity of *Escherichia coli* lipopolysaccharide endotoxin in various animal species. *Can J Micro* 1966;12:1070–1.
23. Novy M, Duffy L, Axthelm M, et al. Experimental primate model for *Ureaplasma* chorioamnionitis and preterm labor. *J Soc Gynecol Investig* 2001;8(Supp 1):48A.(Abstract)
24. Nau GJ, Richmond JF, Schlesinger A, Jennings EG, Lander ES, Young RA. Human macrophage activation programs induced by bacterial pathogens. *Proc Natl Acad Sci U S A* 2002;99:1503–8. [PubMed: 11805289]
25. Marina A, Freudenberg M, Galanos C. Interaction of lipopolysaccharides and lipid A with complement in rats and its relation to endotoxicity. *Infect Immun* 1978;19:875–882. [PubMed: 640734]
26. Blatteis CM, Sehic E, Li S. Afferent pathways of pyrogen signaling. *Ann N Y Acad Sci* 1998;856:95–107. [PubMed: 9917870]
27. Blatteis CM, Sehic E. Cytokines and fever. *Ann N Y Acad Sci* 1998;840:608–18. [PubMed: 9629288]

28. Wong YN, Rossignol D, Rose JR, Kao R, Carter A, Lynn M. Safety, pharmacokinetics, and pharmacodynamics of E5564, a lipid A antagonist, during an ascending single-dose clinical study. *J Clin Pharmacol* 2003;43:735–42. [PubMed: 12856387]
29. Lynn M, Wong YN, Wheeler JL, et al. Extended in vivo pharmacodynamic activity of E5564 in normal volunteers with experimental endotoxemia. *J Pharmacol Exp Ther* 2004;308:175–81. [PubMed: 14566003][corrected]
30. Ziegler EJ, Mccutchan JA, Fierer J, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med* 1982;307:1225–30. [PubMed: 6752708]

Figure 1.

Temporal relationships among LPS inoculation, uterine activity, and amniotic fluid (AF) cytokines and prostaglandins are shown in representative animals from each experimental group. The x-axis represents gestational age in days ranging from the vascular implantation surgery until cesarean section. The y-axis is hourly contraction area (HCA), or the level of amniotic fluid TNF-alpha (orange line) or prostaglandin E2 (blue line). **A:** A representative animal receiving intra-amniotic LPS (red arrow head). **B:** A representative animal receiving intra-amniotic TLR4A (green arrow head) one hour prior to intra-amniotic LPS (red arrow head). In this animal, the intraamniotic LPS administration was repeated at the same dose 7 days later in the absence of TLR4A and again at a higher dose 5 days later, demonstrating moderate recovery of LPS responsiveness.

Figure 2.

Bar height is mean and error bars indicate standard error of the mean. **A:** Uterine activity as measured by the peak HCA following saline, LPS, or LPS + TLR4A infusion. The peak HCA is chosen after averaging HCA over each 24 hour period in the first 7 days following infusion. Peak amniotic fluid prostaglandins (**B**) and cytokines (**C**) in control and experimental groups are also shown.