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**Fusobacterium nucleatum** Induces Fetal Death in Mice via Stimulation of TLR4-Mediated Placental Inflammatory Response

Honggi Liu,* Raymond W. Redline,† and Yiping W. Han²*†

Intrauterine infection plays a pivotal role in preterm birth (PTB) and is characterized by inflammation. Currently, there is no effective therapy available to treat or prevent bacterial-induced PTB. Using *Fusobacterium nucleatum*, a Gram-negative anaerobe frequently associated with PTB, as a model organism, the mechanism of intrauterine infection was investigated. Previously, it was shown that *F. nucleatum* induced preterm and term stillbirth in mice. Fusobacterial-induced placental infection was characterized by localized bacterial colonization, inflammation, and necrosis. In this study, *F. nucleatum* was shown to activate both TLR2 and TLR4 in vitro. In vivo, the fetal death rate was significantly reduced in TLR4-deficient mice (C57BL/6 TLR4<sup>−/−</sup> and C3H/HeJ (TLR4<sup>+/−</sup>)), but not in TLR2-deficient mice (C57BL/6 TLR2<sup>−/−</sup>), following *F. nucleatum* infection. The reduced fetal death in TLR4-deficient mice was accompanied by decreased placental necroinflammatory responses in both C57BL/6 TLR4<sup>−/−</sup> and C3H/HeJ. Decreased bacterial colonization in the placenta was observed in C3H/HeJ, but not in C57BL/6 TLR4<sup>−/−</sup>. These results suggest that inflammation, rather than the bacteria per se, was the likely cause of fetal loss. TLR2 did not appear to be critically involved, as no difference in bacterial colonization, inflammation, or necrosis was observed between C57BL/6 and C57BL/6 TLR2<sup>−/−</sup> mice. A synthetic TLR4 antagonist, TLR4A, significantly reduced fusobacterial-induced fetal death and decidual necrosis without affecting the bacterial colonization in the placentas. TLR4A had no bactericidal activity nor did it affect the birth outcome in sham-infected mice. TLR4A could have promise as an anti-inflammatory agent for the treatment or prevention of bacterial-induced preterm birth. *The Journal of Immunology, 2007, 179: 2501–2508.*

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*Abbreviations used in this paper: PTB, preterm birth; AF, amniotic fluid; hTLR, human TLR; Pam3CSK4, N<sub>4</sub>-palmitoylethyl(N<sub>4</sub>)-[2,3-bis(palmitoyleoxy)-(2, RS)-propyl]-Cys-Ser-Lys<sub>4</sub>.*

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response as an important factor in the pathogenesis of bacterial-induced PTB (12).

TLRs are pattern recognition receptors expressed on mammalian cell membranes that play pivotal roles in the host inflammatory responses (for recent reviews see Refs. 16 and 17). To date, a total of 10 human TLRs (1–10) and 12 murine TLRs (1–9, and 11–13) have been identified, with TLR2, TLR4, and TLR5 identified as key receptors for recognizing bacterial surface components (16, 17). TLR2, acting in conjunction with TLR1 or TLR6, is activated by bacterial lipoprotein, peptidoglycan, lipoteichoic acid, lipoarabinomannan, zymosan, and N-palmitoyl-(S)-[2,3-bis(palmitoyloxy)-(2, R5)-propyl]-cysteine (Pam3Cys) (16). TLR4, in conjunction with MD2 and CD14, is predominantly activated by LPS. TLR5 recognizes bacterial flagellin (16).

In this study, the involvement of TLRs in fusobacterial-induced fetal death in mice was investigated. Because F. nucleatum is nonmotile and lacks flagella, the investigation was focused on TLR2 and TLR4. In vitro analysis demonstrated that F. nucleatum was capable of stimulating host proinflammatory cytokine response via both TLR2 and TLR4. However, in the pregnant murine model only TLR4 appeared to be involved in the inflammatory response to fusobacterial infection. We discovered that a lipid A mimic and TLR4 antagonist, TLR4A, was effective in reducing decidual necrosis and fetal death without affecting bacterial colonization in the placentas. TLR4A is a potential therapeutic target for treating intrauterine infections and improving pregnancy outcome.

Materials and Methods

Bacterial and mouse strains and cell lines

Fusobacterium nucleatum 12230 was maintained as previously described (18). C57BL/6, C3H/HeN, and C3H/HeJ mice were purchased from the National Cancer Institute (Bethesda, MD). C57BL/6 TLR2/− and C57BL/6 TLR4/− mice (19, 20) were obtained from Drs. S. Akira (Osaka University, Osaka, Japan) and A. Hise (Case Western Reserve University, Cleveland, OH). All mice were kept in sterilized filtered-topped cages, fed autoclaved food and water, and handled in a laminar flow hood in a BSL2 room. The animal protocol was approved by the Case Western Reserve University Institutional Animal Care and Use Committee (Cleveland, OH). C57BL/6 TLR4 null, C57BL/6 human TLR2 (hTLR2), and C57BL/6 hTLR4/MD2/CD14 cells were purchased from InvivoGen. The cells were maintained at 37°C in 5% CO₂ in DMEM medium with high glucose (4.5 g/L) (Mediatech) supplemented with 10% FBS (Invitrogen Life Technologies) and the antibiotics (Pam3CSK4; E. coli LPS (C) at varying concentrations. The SD values are expressed as bars above and below the geometric symbols.

In vitro activation of the HEK293 cells

HEK293 or HEK293/hTLR2 and HEK293/hTLR4/MD2/CD14, were seeded into 96-well plates at a density of 50,000 cells/well and allowed to grow overnight. The cells were then incubated with N-palmitoyl-(S)-(2,3-bis(palmitoyloxy)-(2, R5)-propyl)-Cys-Ser-Lys₄ (Pam3CSK4), ultra-pure Escherichia coli LPS, or fresh cultures of F. nucleatum 12230 at the indicated doses followed by a 24-h incubation. The supernatant was harvested and centrifuged and the amount of IL-8 secreted was determined by ELISA.

ELISA

Immunon flat-bottom 96-well microtiter plates (Thermo Electron) were coated with goat anti-human IL-8 Ab (4 μg/ml; R&D System) in 100 μl of coating buffer (0.05 M carbonate buffer (pH 9.6)). After overnight incubation at 4°C, the plates were washed six times with washing buffer, i.e., 0.05% Tween 20 in PBS (Sigma-Aldrich), followed by an overnight incubation in blocking buffer (1% BSA in PBS) at 4°C. The plates were washed four times before an aliquot of 100 μl of diluted mammalian cell culture supernatant or human IL-8 (R&D Systems) were added to each well and incubated overnight at 4°C. The plates were washed, followed by 3-h incubation with polyclonal rabbit anti-human IL-8 Ab (1/1000; Endogen) at room temperature. After the plates were washed, a HRP-conjugated goat-anti-rabbit IgG Ab (1/2500; BioSource International) was added and incubated for 1.5 h at room temperature. After the final wash, 100 μl of tetramethylbenzidine (Pierce) was added to each well. The color reaction was stopped by adding 100 μl of 2 M sulfuric acid. The light absorbance was measured at 450 nm on a Bio-Rad Model 680 microplate reader. The experiment was performed in triplicate and repeated multiple times.

Mating, i.v. injection of mice and kinetics of infection

Mating and i.v. injection of F. nucleatum 12230 were conducted as previously described (12). Briefly, 10-wk-old mice were caged together at a female-to-male ratio of 2:1. Mating was determined by the presence of a white vaginal plug. The day when the plug was detected was termed day 1 of gestation. Pregnant mice were infected on day 16 or 17 of gestation. Cultures of F. nucleatum 12230 were washed once with sterile PBS. Based on its OD at 600 nm, the cultures were adjusted so that the estimated titer was ~10⁶ CFU/ml. The actual CFU was determined by plating serial dilutions of the culture suspension onto blood agar plates. An aliquot of 100 μl of the bacterial suspension was injected into the tail vein of each mouse. The birth outcome was recorded. For kinetics of infection, groups of 4–16 mice were sacrificed at each indicated time point. The liver, spleen, and placenta were harvested from each pregnant mouse, weighed, and homogenized in sterile PBS. Full thickness cross-sections of the placenta and underlying uterus were obtained on 2–5 placentas per animal in each experimental group. The live bacterial titer was determined by plating serial dilutions on blood agar plates. The bacterial titer was expressed as log₅ CFU/gram tissue. In the TLR4A treatment experiment, ~1 × 10⁶ CFU of F. nucleatum 12230 in 100 μl of PBS were mixed with either 0.1 mg of TLR4A in 100 μl of vehicle (2% glycerol) or 100 μl of 2% glycerol and injected through tail veins on day 16 of gestation. On day 17, a second dose of 0.1 mg of TLR4A in 100 μl of 2% glycerol or 100 μl of 2%
glycerol alone was injected. For controls, TLR4A or its vehicle was injected into pregnant CF-1 in the absence of *F. nucleatum* 12230.

**Histopathological analysis of infected fetoplacental units**

The histopathological analysis was conducted as previously described (12). Full thickness H&E-stained sections were examined in a blinded fashion by a pathologist (R.W.R.) as before (12). For each specimen, necrosis and inflammation (polymorphonuclear leukocytes) were evaluated in three decidual regions (margin, center, and paracentral venous sinusoidal), three placental regions (spongiotrophoblast, labyrinth, and chorioallantoic plate), and two regions of the placental membranes (yolk sac and amnion) (12). Data are presented as the group mean ± SD of the percentage of positive regions divided by the total number of regions sampled for each mouse.

**Statistical analysis**

All results are expressed as the mean value ± SD. The nonparametric Mann-Whitney *U* test was used for comparison of fetal death rates and the decidual necrosis, and the Student *t* test was used for the bacterial titers (SPSS 12.0.1 for Windows). Differences between groups were considered significant with *p* < 0.05.

**Results**

**F. nucleatum** 12230 stimulates IL-8 expression via human TLR2 and TLR4 in vitro

Previous work showed that *F. nucleatum* 12230 stimulated IL-8 expression from epithelial cells. To examine the involvement of TLRs in the activation of the proinflammatory response, the ability of *F. nucleatum* to stimulate IL-8 expression was tested using HEK293 null cells, which lack TLR2 or TLR4, and HEK293 cells stably transfected with human TLR2 or TLR4/MD2/CD14. The expression of TLR2 and TLR4 in the stably transfected cells was verified by reverse-transcription PCR and flow cytometry using TLR2- or TLR4-specific Abs (data not shown).

*F. nucleatum* 12230 was incubated with the 293 cells at varying multiplicities of infection (MOI; bacteria:HEK cells) for 24 h. The amount of IL-8 secreted into the culture medium was determined by ELISA. At MOI 50, *F. nucleatum* stimulated little or no IL-8 from HEK293-null cells but did so from both HEK293/TLR2 and HEK293/TLR4/MD2/CD14 cells in a dose-dependent manner (Fig. 1A). The induction of TLR-transfected cells reached a plateau at MOI 50, with the maximum concentration of IL-8 at between

**FIGURE 2.** Birth outcome of different mouse strains in response to i.v. infection of *F. nucleatum* 12230. Approximately 3–7 × 10⁷ CFU were injected into each pregnant mouse. The fetal death rate was expressed as the percentage of dead fetuses from the total number of pups born to each mother. Each geometric symbol represents one pregnant mouse. A, C57BL/6 (○; n = 7), C57BL/6 TLR2<sup>−/−</sup> (△, n = 10), and C57BL/6 TLR4<sup>−/−</sup> (□; n = 9). B, C3H/HeN (●; n = 9) and C3H/HeJ (■; n = 13). The horizontal lines indicate the average fetal death rates.

**FIGURE 3.** Kinetics of infection in the liver, spleen, and placenta of different mouse strains following the i.v. injection of *F. nucleatum* 12230. The injection doses for each strain at each time point were also shown. Approximately 3–7 × 10⁷ CFU were injected into each pregnant mouse. The live bacterial titers in each organ at 6, 16 (or 18), and 48 h postbacterial infection were expressed as log<sub>10</sub> (CFU/gram tissue). A, C3H/HeN (■), n = 11 (6 h), 8 (18 h), and 7 (48 h); C3H/HeJ (□), n = 8 (6 h), 7 (18 h), and 6 (48 h). B, C57BL/6 (●), n = 7 (6 h), 8 (16 h), and 7 (48 h); C57BL/6 TLR2<sup>−/−</sup> (□), n = 7 (6 h), 16 (16 h), and 5 (48 h); and C57BL/6 TLR4<sup>−/−</sup> (△), n = 4 (6 h), 9 (16 h), and 8 (48 h). The SD is labeled above each bar. The asterisks indicate a significant difference of the bacterial titers between the wild-type and the mutant mice (*, * p < 0.05).
The induction of HEK293 null cells increased slowly through a MOI of 200, with a maximum IL-8 concentration of 1,000 pg/ml (Fig. 1A). The TLR2-specific ligand Pam3CSK4 and the TLR4-specific ligand E. coli LPS only activated their respective receptors but not vice versa, indicating the specificity of the TLR activation (Fig. 1, B and C). Taken together, these results indicate that although the expression of IL-8 in response to F. nucleatum may involve TLR-independent pathway(s), it was much enhanced in the presence of human TLR2 or TLR4.

Fetal death rate was reduced in TLR4-deficient but not in TLR2-deficient mice in response to F. nucleatum 12230 infection

To assess the involvement of TLR2 and TLR4 in bacterial-induced fetal death, two knockout strains, C57BL/6 TLR2−/− and C57BL/6 TLR4−/−, and one strain of spontaneous TLR4-deficient mice, C3H/HeJ, which carries a point mutation in the TLR4 gene, were tested along with their respective wild-type strains, C57BL/6 and C3H/HeN. An aliquot of CFU (3–7 × 10⁷) of F. nucleatum 12230 were injected into each pregnant mouse through the tail vein on day 16 of gestation. Statistical analysis
presented improved birth outcome following To investigate why TLR4-deficient but not TLR2-deficient mice deficient C3H and C57BL/6 mice HeN and 8.8% in C3H/HeJ ( ) with a fetal death rate of 51.4% in C3H/HeJ mice (Fig. 2B). Similar differences also existed between C3H/HeN and C57BL/6 TLR4 mice (Fig. 2B) with a fetal death rate of 51.4% in C3H/HeN and 8.8% in C3H/HeJ (p < 0.05). Extent of F. nucleatum colonization differs in TLR4-deficient C3H and C57BL/6 mice To investigate why TLR4-deficient but not TLR2-deficient mice presented improved birth outcome following F. nucleatum infection, the live bacterial titers in the liver, spleen, and placenta of the infected mice were determined at 6, 16 (or 18), and 48 h postinfection. Again, no difference was detected between the bacterial dosages injected into each mouse strain (data not shown). High fetal death rate was observed in C57BL/6 and C57BL/6 TLR2−/−, i.e., 81.4 and 78.7%, respectively (Fig. 2A). However, a significantly lower fetal death rate of 3.7% was observed in C57BL/6 TLR4−/− mice (Fig. 2B). F. nucleatum colonization in the C3H and C57BL/6 background. Alternatively, mice with total deletion of TLR-4 may have compensatory adaptations not observed in those with point mutations. TLR4 deficiency led to reduced necroinflammatory response in both C3H and C57BL/6 backgrounds To determine the role of TLR2 and TLR4 in the placental inflammatory response to fusobacterial infection, placentas from C3H/HeN and C3H/HeJ mice were collected at 18 and 48 h postinfection, and from C57BL/6, C57BL/6 TLR2−/−, and C57BL/6 TLR4−/− mice at 48 h postinfection, all from the same mice from which the bacterial titers were determined, followed by histopathological analysis. Necrosis and inflammation were apparent in the decidua, the marginal region, the labyrinth, and the membranous yolk sac of the placentas from C3H/HeN and C57BL/6 (Fig. 4). The inflammatory infiltrate was composed almost entirely of polymorphonuclear leukocytes (Fig. 4, inset). However, they were reduced or lacking in those from the TLR4-deficient C3H/HeJ and kinetics of infection was detected between C57BL/6 TLR2−/−, C57BL/6 TLR4−/−, and C57BL/6 mice (Fig. 3B). In contrast, a significant difference was observed between C3H/HeN and C3H/HeJ mice (Fig. 3A). F. nucleatum 12230 colonized less in the placenta and spleen in HeJ than in HeN at 6 h postinfection (p < 0.05). It also proliferated to a lesser extent in the placenta after 18 and 48 h, respectively (p < 0.05). These results suggest that TLR4 may play different roles in F. nucleatum colonization in the C3H and C57BL/6 background. Extent of F. nucleatum colonization in mice infected with F. nucleatum is shown in the Table I. Summary of placental histopathological lesions of TLR4−/− and TLR4-deficient mice infected with F. nucleatum 12230% Duration of infection (h) No. mothers/no. placentas Percentage placentas positive for Extent of F. nucleatum 12230 colonization differs in TLR4- deficient C3H and C57BL/6 mice FIGURE 5. Effect of TLR4A on the birth outcome of outbred CF-1 mice in response to F. nucleatum infection. On day 16, each pregnant CF-1 mouse received 1 × 10⁹ CFU of F. nucleatum 12230 plus 100 µl of 2% glycerol (vehicle) ( ; n = 16), 1 × 10⁹ CFU of F. nucleatum 12230 plus 0.1 mg of TLR4A in 100 µl of 2% glycerol ( ; n = 14), PBS plus 0.1 mg of TLR4A in 100 µl of 2% glycerol ( ; n = 4), or PBS plus 100 µl of 2% glycerol (vehicle) ( ; n = 2). On day 17, each mouse received a second dose of TLR4A or 2% glycerol as on the day before. The fetal death rate was expressed as percent dead fetuses of the total number of pups born to each mother. The horizontal lines indicate the average fetal death rate of each group. Fn, F. nucleatum 12230. FIGURE 6. Effect of TLR4A on decidual necrosis in response to fusobacterial infection. Placentas in the TLR4A-treated group (□) and a placebo group (□) were harvested 48 h after F. nucleatum (Fn) infection. The percentage of placentas with necrosis in three different regions (central, paracentral, and marginal) in the decidua was calculated respectively for each pregnant mouse. Each bar represents the mean value for each group in each decidual region. The SD is labeled above each bar. * Number of pregnant mice tested in each group. The asterisks indicate significant difference between the treatment and placebo group ( , p < 0.05).
C57BL/6 TLR4−/− mice despite a similar initial inoculum of F. nucleatum (Fig. 4). When the percentage of placenta with any necrosis or inflammation was calculated in different regions, the calculated difference between the wild-type and the TLR4-deficient mice was significant (p < 0.05; Table I). These results indicate that TLR4 played a pivotal role in the placental necroinflammatory response to F. nucleatum infection in both C57BL6 and C3H1 mice. In contrast, C57BL6 TLR2−/− mice showed similar histopathology as C57BL6/mice (Table I), suggesting the lack of TLR2 involvement in the placental inflammatory response. This was consistent with the high fetal death rate observed in the C57BL6 TLR2−/− mice.

TLR4 antagonist TLR4A reduced fetal death in CF-1 mice without affecting the bacterial colonization in the placenta

Synthetic TLR4A is a lipid A mimic and an antagonist of TLR4 (21). To further test the role of TLR4 in F. nucleatum-induced fetal death, an aliquot of 100 μg of TLR4A or an equal volume of the compound vehicle was coinjected with 1 × 10⁶ CFU of bacteria into each pregnant outbred CF-1 mouse on day 16. On day 17, another dose of 100 μg of TLR4A or an equal volume of the compound vehicle was injected. The fetal death rate of the group receiving the compound vehicle and F. nucleatum was 92.5% while that of the group receiving TLR4A in 100 μl of 2% glycerol (vehicle) (Fig. 7; Table I). These results suggest that T. nucleatum is capable of activating multiple pattern recognition receptors, including but not limited to TLR2 and TLR4. Expression of both TLR2 and TLR4 has been reported before in other murine infection models. For instance, the activation of TLR4 in the trophoblastic cells induced cytokine production, the activation of TLR2 induced apoptosis (24). The current study also suggests different roles for TLR2 and TLR4 in murine pregnancy. At the dosage tested F. nucleatum 12230 induced significantly reduced fetal death and placental inflammation in TLR4-deficient mice in both the C57BL6 and C3H backgrounds as compared with the TLR4+/− mice. The fact that the reduction of fetal death was observed in two different TLR4-deficient mouse strains confirmed the attribution to TLR4 rather than to the different genetic backgrounds of the strains (see below). The current study did not find TLR2 to be critically involved in the pathogenesis of F. nucleatum-induced intrauterine infection in mice. No significant difference was observed between the wild-type and TLR2-deficient mice in terms of the fetal death rate, bacterial colonization, or placental inflammatory response. This could be due to the lack of TLR2 expression in the mouse placenta (25). Whether or not TLR2 plays a role in adverse pregnancy outcome in humans needs further investigation.

The improved birth outcome in the TLR4 deficient mice could be due to one or both of the following mechanisms: 1) the inhibition of bacterial colonization and proliferation in the mouse placentas; and 2) the reduction of the inflammatory response to bacterial infection. Assessing the relationship between bacterial titers and necroinflammatory changes in animals allowed us to test both of these scenarios. In both the C57BL6 and C3H mice F. nucleatum colonized within the placenta without causing systemic infections (Fig. 3), similarly as previously reported in the outbred mice (12). Interestingly, in C3H/HeJ mice fusobacterial colonization was significantly reduced in the placenta and spleen compared with in C3H/HeN, suggesting that the TLR4 deficiency affected bacterial colonization (Fig. 3). This contrasted with the previous observations that Leptospira and E. coli colonized more in the liver in C3H/HeJ than in the wild-type C3H mice (26, 27). In the C57BL6 mice, however, neither TLR2 nor TLR4 deficiency exhibited an effect on fusobacterial colonization (Fig. 3). The discrepancy observed between C3H and C57BL6 mice may be due to the different genetic backgrounds of the strains, which has been reported before in other murine infection models. For instance, the BALB/c IL-12 knockout mice were highly susceptible to infection by Helicobacter pylori, but the C57BL6 IL-12 knockout mice were resistant to infection by the same organism (28).
Immunohistochemical staining (data not shown) verified our previous data showing that necrosis and inflammation colocalized with F. nucleatum colonization in the placenta and uterus of genetically intact mice. These inflammatory changes would at least in part be expected to depend on the engagement of local TLRs on placental cells by pathogen-associated molecular patterns expressed by F. nucleatum and might be decreased in mice lacking the expression of specific TLRs. We observed that irrespective of the genetic background, TLR4 deficiency resulted in reduced necroinflammatory response to fusobacterial infection in the placenta (Fig. 4 and Table I). These results suggest that TLR4 promoted fetal death through stimulation of the inflammatory response rather than by influencing the bacterial colonization.

The validity of this conclusion was further tested by using the synthetic TLR4 antagonist TLR4A, which had been shown to prevent the expression of proinflammatory genes and reduce inflammatory bowel disease in mice (21). The injection of TLR4A into F. nucleatum-infected pregnant CF-1 mice led to a near 2-fold reduction in the fetal death rate (Fig. 5). Outbred mice were chosen for this test so that the effects of the genetic background of different inbred strains could be eliminated. TLR4A exhibited no bactericidal effect in vitro. F. nucleatum colonized the placentas to a similar extent in both the treatment and the control group. Thus, the reduction of fetal death was not caused by the inhibition of bacterial colonization or the killing of the bacteria. Histopathological analysis showed a significant reduction of decidua necrosis in response to fusobacterial infection in the TLR4A treatment group compared with the placebo group (p = 0.01), confirming that TLR4A reduced the necroinflammatory response.

It should be pointed out that, under our test conditions, fetal death was reduced by TLR4A treatment but it was not eliminated or reduced to a similar extent as that seen in the TLR4-deficient mice. Two nonmutually exclusive possibilities exist. The first possibility is that TLR4A did not completely block TLR4 activation. Two consecutive doses of 0.1 mg of TLR4A per pregnant mouse per injection were administered. It is not known whether both dosages were necessary and whether higher doses or more frequent injections would improve the birth outcome further. The second possibility is that F. nucleatum may contribute to fetal death through additional TLR4-independent pathways.

The discovery that TLR4A treatment improves pregnancy outcome in mice is significant in several ways. First, it confirmed that the placental inflammatory response plays a key role in murine fusobacterial infection, similar to the intrauterine infection in humans. Thus, it validates the use of the pregnant murine model to study the pathogenesis of fusobacterial-induced adverse pregnancy outcome. Second, because placental TLR4 expression has been shown to increase in women with PTB and chorioamnionitis compared with those with PTB but without chorioamnionitis (29), TLR4A may be useful in the treatment of these infections in humans. Third, the results indicate the importance of bacterial LPS in the pathogenesis in intrauterine infection. TLR4A may be useful for the treatment of infections caused by other Gram-negative organisms. Fourth, our results may explain at least in part why antibiotic therapies have not been successful at reducing the preterm birth rate (30–32). Although antibiotics may kill the bacteria, they are unable to eradicate the dead microorganisms that are capable of inducing inflammatory responses. Based on our findings, antibiotic therapies in conjunction with anti-inflammatory therapies are more likely to succeed in improving birth outcome than either therapy alone.

The efficacy of TLR4A in this murine model adds to the scarce arsenal of potential agents for the prevention of PTB. Although 17-α hydroxyprogesterone caproate has been used in pregnant women with a prior PTB history, this compound was recently found to cause significant maternal morbidity in mice (33). TLR4A, in contrast, did not exhibit any detectable adverse effect on either the fetus or the mother in our study. Thus, it is promising anti-inflammatory agent for the treatment and prevention of adverse pregnancy outcomes. As indicated above, because TLR4A is nonbactericidal, it probably will achieve a maximal protective effect as an adjunct to specific antimicrobial therapy. The feasibility of using TLR4A in humans needs further investigation.

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Disclosures

The authors have no financial conflict of interest.

References


CORRECTIONS


The fourth author, who provided the clone 20/70 rat anti-mouse IgG Ab for this paper, is retracting his authorship from this article. Dr. Jörg Zwirner was not informed before manuscript submission that he would be included as an author. All authors accept this decision, which is the result of an agreement mediated by the Ombudsman of the German Research Council.

In References, Dr. Zwirner’s last name is misspelled. The corrected Ref. 23 is shown below.


The first author’s first and last names are transposed. The correct name is Mustapha Oumouna.


In Fig. 1, dimension bars in A and the symbol key in B were omitted. The two bottom left panels of Fig. 1A have been replaced; the results and conclusions of the paper remain unchanged. The corrected figure is shown below.

In Fig. 4, the labels are missing in the last four panels of B. The corrected figure is shown below.

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One of the first author’s institutional affiliations was omitted. The corrected author and affiliation lines are shown below.

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