Published in final edited form as:

Anaerobe. 2008 November ; 14(5): 256–260. doi:10.1016/j.anaerobe.2008.08.002.

***Prevotella bivia* as a Source of Lipopolysaccharide in the Vagina**

Alla Aroutcheva, MD, PhD¹, Zaodung Ling, PhD², and Sebastian Faro, MD, PhD³

¹ Rush University Medical School/Stroger Hospital of Cook County, 1901 W. Harrison Str, Chicago, IL

² Rush University College of Pharmacology, 1653 W Congress Pkw, Chicago, IL

³ Women's Hospital of Texas, 7400 Fannin, Houston, TX

Abstract

Objectives—To compare vaginal lipopolysaccharides (LPS) concentrations between patients with and without bacterial vaginosis (BV), to evaluate the correlation between *Prevotella bivia* colonization density and LPS concentration, and to determine the impact of LPS on loss of dopamine neurons (DA).

Methods—Vaginal washes obtained from patients with (n=43) and without (n=59) BV were tested for quantity of *P. bivia* cells using quantitative PCR and for concentrations of LPS using the *Limulus* Amebocyte Lysate gel clot method. *Prevotella bivia*, *Gardnerella vaginalis* and *Escherichia coli* sonicated cell extracts were also tested for LPS production. DA neuron cells obtained from embryonic day (E) 14.5 pregnant rats were exposed to fluid from eight vaginal washes; tyrosine hydrolase immunoreactive staining was applied for visualization and cell counts.

Results—The median LPS concentrations were dramatically higher among patients who had symptoms of BV compared to those who did not have symptoms (3235.0 vs 46.4 EU/ml, respectively, $P<0.001$); patients who had BV also had much higher colonization densities of *P. bivia* (0.06 ± 0.36 vs 5.4 ± 2.2 log₁₀ CFU/ml, respectively, $P<0.001$).

Prevotella bivia cell lysates resulted in a higher LPS concentration ($10,713.0\pm306.6$ EU/ml) than either *E. coli* ($4,679.0\pm 585.3$ EU/ml) or *G. vaginalis* (0.07 ± 0.01 EU/ml of LPS).

The loss of DA neuron was 20–27% in cultures treated with vaginal washes from BV-negative patients and 58–97% in cultures treated with vaginal washes from patients with BV.

Conclusion—*P. bivia* produces high LPS concentration, which may create a toxic vaginal environment that damages DA neurons.

Keywords

Bacterial vaginosis; *Prevotella bivia*; *Gardnerella vaginalis*; *E. coli* Lipopolysaccharides; Dopamine neurons; rat model

Corresponding author: Alla Aroutcheva, MD, PhD, Stroger Hospital of Cook County, Division of Infectious Diseases, 1901 W Harrison Str. #LL 715, Phone: (312) 864-7053, Fax: (312) 864- 9522, E-mail: aaroutch@rush.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1.1. Introduction

The gram-negative bacterial vaginosis (BV)-associated anaerobe, *Prevotella bivia*, inhabits the lower genital tract and has the propensity for adherence to and invasion of human cervix epithelial cells [1]. Anaerobic bacteria were isolated in 91% and 18% of BV-positive and BV-negative patients, respectively[2]; *Prevotella bivia* and other *Prevotella* spp. represented 44% of all anaerobes isolated in the BV-positive group. Evaluation of BV with gram-stained vaginal slides showed that *Prevotella* and *Gardnerella vaginalis* counts correlated strongly with the Nugent score [3].

It has been shown that an increased rate of premature labor and preterm delivery [1–3] occurs among women with *P. bivia* at a concentration of $>10^4$ bacteria/ml of vaginal fluid [4]. An experimental rabbit model also confirmed an association between *P. bivia* and preterm birth, increased level of TNF- α , and chronic intrauterine and fetal infection [5]. A persistent intrauterine inflammatory state that can occur in chronic conditions such as BV suggests that preterm delivery may follow chronic exposure to organisms, perhaps with resulting fetal brain damage [6,7].

Prevotella bivia and other *Prevotella* species contain endotoxin, lipopolysaccharides (LPS), on their outer membranes; LPS is the most potent antigenic component of the Gram-negative bacterial cell wall. Some species of *Prevotella* produce LPS that is much more potent in inducing a rapid platelet response than the LPS found in *Escherichia coli* and *Salmonella typhimurium*. [8].

Exposure to a low dose of the bacteriotoxin, LPS, during a critical window of vulnerability of fetal development led to the birth of rat pups with fewer than normal dopamine (DA) neurons [9,10].

Since BV results in a relatively high concentration of LPS-producing *P. bivia* in vaginal fluid, the aim of this study was to determine the correlation between the quantity of *P. bivia* and the level of LPS in the vagina of patients with BV. We also investigated whether LPS presented in the vaginal fluid of patients with BV is toxic for dopamine neurons.

2. 1. Material and Methods

2.1.1. Vaginal wash

A total of 102 vaginal washes (43 from patients with BV and 59 from patients without BV) were obtained from women of reproductive age visiting the Women's Hospital of Texas, Houston, TX. The samples were studied for the level of endotoxin, lipopolysaccharides (LPS) and the quantity of *P. bivia* in the vagina. All patients enrolled into the study signed written informed consent. The study had approval from the Woman's Hospital of Texas IRB committee.

BV was diagnosed based on clinical characteristics (Amsel criteria) [11] and on interpretation of a gram-stained slides, as described by Nugent et al [12]. Vaginal washes were conducted by instilling 3 ml of sterile non-pyrogenic saline into the vagina and aspirating the fluid with a sterile syringe. The process was repeated using the saline aliquot three times. The sample was maintained at -20°C and shipped to the laboratory packed in dry ice. Before testing washes were centrifuged at $11,000g \times 15\text{ min}$ to separate cells. Supernatant was used for LPS detection and the cell pellet was used for extraction of DNA.

2.1.2. LPS determination

LPS was measured using the *Limulus* Amebocyte Lysate (LAL) (Cape Cod Inc., East Falmouth, MA) in pyrotell gel clot method according to manufacturer's instruction. Briefly, supernatant from vaginal washes and bacterial cell walls was diluted two fold using endotoxin free water and tubes (Cape Cod Inc.). Then 0.1 ml (v/v) of LAL reagent was added to each tube. After 60 min of incubation in a water bath at 37°C, the tubes were removed and gently inverted. If gel had formed and remained intact at the bottom of the tube, the test was evaluated as positive. The concentration of endotoxin in the sample was calculated by multiplying the dilution factor on LAL-positive tests; the lowest concentration that can be determined using the gel clot technique is 0.03 LPS EU/ml. *Escherichia coli* endotoxin (Cape Cod Inc.) was used as a control.

To compare LPS production by gram-negative vaginal microorganisms, four strains of each microorganisms, *P. bivia*, *G. vaginalis* and *E. coli* were tested. *Prevotella bivia* and *G. vaginalis* were isolated from study patients in the Microbiology Laboratory of Women's Hospital of Texas and four vaginal strains of *E. coli* were selected from our culture collection; no patients with vaginal *E. coli* were found in the current study group.

Cells were harvested from agar plates after 24hrs of incubation and suspended in endotoxin free water (Cape Cod Inc.) to achieve 0.5 McFarland turbidity, that corresponds to 1.5×10^8 CFU/ml. The suspension was then diluted 10-fold to a concentration of 10^7 CFU/ml. Cell walls were disrupted using sonication (15 sec $\times$ 3 times). Lysate was separated from cell debris by centrifugation and then tested for LPS concentration.

2.1.3. DNA isolation

The vaginal wash pellets containing squamous epithelial and bacterial cells were used for DNA isolation. DNA was isolated using the Bactozol kit (Molecular Research Center, Inc., Cincinnati, OH). Briefly, cell suspension was treated with 0.1ml of 1X Bactozyme and incubated at 50°C for 60 min. After lysis was achieved, we used DNAzol and polyacrilamid to isolate DNA. The DNA was precipitated by adding 100% ethanol.

The total DNA concentration at A 260 and purity at ratio A 260/A280 was measured using a Beckman DU-600 spectrophotometer.

2.1.4. Quantitative PCR

For sequencing and amplification of the *Prevotella bivia* 16SrRNA gene, the following primers were used: forward: 5'-AGGGATAACCCACCGAAAGTTGGA-3', reverse: 5'-TAAATCCGGATAACGCCGAACCT-3'. Selected sequences were analyzed for homology using the GeneBank database with BLAST web site (National Center of Biotechnology Information, National Institute of Health, Bethesda, MD). All primers were obtained from IDT, Coralville, IA.

Quantitative PCR was performed using a Light Cycler 143 (Roche, Indianapolis, IN) and SYBR green, as a fluorescents dye (Takara Bio Inc., Otsu Shiga, Japan). After 95°C 30sec of denaturation, the shuttle PCR protocol involved 35 cycles of 95°C 5 sec and 60°C 20 sec. Purified genomic DNA from *P. bivia* ATCC 29303 was used to generate a standard curve consisting of 10^3 to 10^7 copies of DNA [13]. The bacterial counts represent the total amount in 1 ml of vaginal wash. This was obtained by determining the total DNA isolated from the wash samples times the number of bacteria in 50 ng of DNA.

2.1.5. Dopamine neuron cell culture bioassay

Eight vaginal washes collected from five patients with BV and three patients with normal vaginal ecosystem were employed for the DA toxicity study. Vaginal washes collected from

women with BV contained $\log_{10} 6.64 \pm 0.73$ of *P. bivia* cells/ml and LPS at a level of 8072 EU/ml (2 patients) and of 5030 EU/ml (1 patient); two other washes contained $\log_{10} 6.10$ of *P. bivia* cells/ml and 2048 EU/ml of LPS. Three patients without BV had no *P. bivia* in the vagina and LPS at a level of 0 EU/ml (2 patients) and of 16 EU/ml (1 patient). DA neuron tissue culture was obtained from embryonic day (E) 14.5 pregnant rats. Procedures used in these studies were approved by the Institutional Animal Care and Utilization Committee (IACUC) of Rush University. The protocol for this in vitro study has been published elsewhere [14]. The number of tyrosine hydrolase (TH) immunoreactive cells assessed using procedure is described in detail elsewhere [15]. Briefly, the single cell suspension was prepared from mesencephalic tissue using sequential incubation with trypsin and DNAase/trypsin inhibitors. The tissue was triturated into single cell suspension in 3 ml complete media and plated into the 48-well plate. The plate was incubated for 24 hrs to stabilize DA neurons. The following day, 20% of the media was replaced with vaginal wash fluid for an additional 72 hrs incubation. The plate was washed with PBS, fixed with 4% formaldehyde, and prepared for immunocytochemistry with (TH) stain, the traditional marker for DA neurons. The plate was incubated with blocking solution containing 0.25% Triton-X and normal horse serum for one hour. Without washing, mouse anti-rat TH antibody (Immunostar, Stillwater MN; 1:20,000) was added and the plate was incubated at 4°C overnight. The endogenous peroxidase activity was eliminated using a 1-h incubation with 0.1% periodate. The immunohistochemical procedure was continued by using a biotinylated horse anti-mouse IgG (0.5%; Vector Laboratories, Burlingame CA) for 1 h and peroxidase conjugated avidin–biotin complex (Vector Laboratories) for 1 h. The TH immunoreactive cells were visualized using 0.05% 3,3'-diaminobenzidine (DAB), 0.5% nickel sulfate, and 0.003% H_2O_2 in I/A solution (10 mM imidazole/50 mM sodium acetate).

2.1.6. Statistical analysis

Categorical variables were tested by performing the chi-square test. We graphically displayed the distribution of *P. bivia* colonization density and the LPS concentration as a scatter plot and evaluated the correlation of these two factors by calculating Spearman's rho. For all analyses we used a P-value of 0.01 to indicate statistical significance. Analyses were performed using Stata version 9.2 (Stata Inc., College Station, TX.).

3.1. Results

3.1.1. *Prevotella bivia* and LPS in the vagina of patients with and without BV

As shown in table 1, patients who had BV were much more likely to have vaginal colonization by *P. bivia* compared to patients who did not have BV (90.7% vs 3.4%) with the mean colonization density $\log_{10} 5.4$ vs 0.06 CFU/ml, $P < 0.001$. The LPS concentration also discriminated between patients with and without BV (3235.0 vs 46.4 EU/ml, $P < 0.001$). Surprisingly, *P. bivia* cells were not found in the vagina of five BV-positive patients, which correlated with zero amounts of LPS in three patients and low levels of LPS (6.125 – 26.5 EU/ml) in two patients.

There was strong correlation between the *P. bivia* colonization density and the LPS concentration (Spearman's rho = 0.9750, figure 1).

Setting a cutpoint of $\log_{10} 2$ of *P. bivia* and a level of LPS of 128 EU/ml, we showed that patients who had BV were much more likely to have a high density of *P. bivia* and LPS concentration than those without BV (Tables 2 and 3).

3.1.2. Comparison of level of LPS produced by tested gram-negative microorganisms

Production of LPS by the mean count of cells $\log_{10}$ 7.1/ml for *P. bivia*, *G. vaginalis* and *E. coli* were different. *Prevotella bivia* produced $10,700 \pm 307.0$, whereas the same numbers of *E. coli* cells produced less than half that amount of LPS ($4,700 \pm 585.1$ EU/ml). A trace amount of LPS (0.07 ± 0.01 EU/ml) was observed from the *G. vaginalis* lysates.

3.1.3. Effect of vaginal washes with different level of LPS on loss of DA neurons in cell culture

Endotoxin levels correlated positively with DA neuron loss ($P=0.006$). The loss of DA neurons in cultures to which washes from normal vaginal environments were added was 20% (two patients with no LPS found) and 27% (one patient with LPS level 16 EU/ml), while DA neuron losses in the cultures to which vaginal washes from BV patients were added were 58% (one patient with LPS level 2048 EU/ml), 84% (two patients with LPS level 5030 EU/ml), and 97% (two patients with LPS level 8072 EU/ml).

4.1. Discussion

The current study showed that altered vaginal microecology creates a toxic environment in the lower genital tract of women. Patients with *E. coli* as a source of LPS were excluded from this study. Elevated numbers of *P. bivia* in vaginal fluid showed that this Gram negative anaerobe might be a possible source of LPS in the vagina. Recently published data showed that vaginal fluid of healthy microecology contains twenty different aerobic and anaerobic bacterial flora but no *P. bivia* [16].

Among BV-associated microorganisms, *G. vaginalis* is the most frequently found in the vagina of patients with bacterial vaginosis [17]. However, compared to *P. bivia*, *G. vaginalis* produces an extremely minimal amount of LPS. These microorganisms have a symbiotic relationship [18][16] and were found growing together in 59.1% of women with BV compared to 3.9% of women without BV [19]. When a mixture of facultative anaerobes and *P. bivia* were grown together, the pathogenicity of *P. bivia* increased [20]. *Prevotella bivia* and *G. vaginalis* were detected in a high rate in patients with cervical cancer [21].

The views about LPS production by *G. vaginalis* vary. Sadhu et al. showed that *G. vaginalis* has a unique cell wall structure, resembling that of gram-positive bacteria, but with an unusually thin structure [22]. Using an LAL test these researchers showed that LPS was produced by *G. vaginalis*, albeit in low amounts. Greenwood and Picket described *G. vaginalis* as more closely resembling gram-negative bacteria. They also found cell wall material of *G. vaginalis* positive for endotoxin, but only very high numbers of cells were studied [23].

P. bivia has been isolated from the blood of patients delivered by caesarian section [24] as well as those diagnosed with acute pelvic inflammatory diseases [25]. It has been shown to have the capability to invade the human cervix [1] and cause intrauterine infection [20]. These reasons make this organism a risk factor for pregnancy outcome and fetus development. Our study findings suggest that the concentrations of *P. bivia* and LPS in the vaginal fluid may contribute to the severity of BV and to the development of complications in pregnancy, such as preterm labor, premature rupture of membranes, postpartum endometritis, and postoperative pelvic infection [26,27].

Microbial attack of the fetus takes place in approximately 10% of pregnancies with intra-amniotic infection, and the human fetus is capable of deploying an inflammatory response (cellular and humoral) in the mid-trimester of pregnancy [28].

LPS is the most potent antigenic component of the gram-negative bacterial cell wall and modulates the expression of various pro-inflammatory cytokines, activates production of

lysosomal enzymes, and increases phagocytosis [29]. Purified endotoxin from *P. bivia* is clearly able to induce a cytokine response in monocytic cells [30].

The elevated concentration of LPS in the vaginal fluid and cervical mucus of pregnant women with BV was reported by Platz-Cristensen et al. [31]. High amounts of LPS in the vaginal environment can have numerous consequences. It was demonstrated that the toll-like receptor-4 (TLR-4) plays a role in lipopolysaccharide-induced preterm birth in mice [32]. Antenatal exposure to intra-amniotic endotoxin initiates a complex series of events including an inflammatory cascade that can alter lung structure [33]. An animal study showed that LPS causes histopathological changes in the reproductive organs of pregnant animals [34].

While BV has been considered a relatively benign problem, it can be a risk factor for the offspring and contribute to the risk of long-term adverse neurological outcomes, such as hyperactivity, academic difficulties in school, and severe handicaps, including cerebral palsy and periventricular leukomalacia.[35–38].

Maternal LPS triggers cytokine production by fetal tissue and even in low doses can dramatically sensitize the immature brain to injury [39–41].

Periventricular leukomalacia (PVL), the dominant form of brain injury in premature infants, is characterized by diffuse white matter injury and is associated with cerebral palsy. Clinically, cerebral palsy and periventricular leukomalacia are associated with intrauterine infection and inflammation[37,42]. Animal models help to understand mechanism of the brain injury [43–45]. Induction of maternal bacterial infection in rabbits can reproduce a white matter injury in the fetal rabbit brain that has some similarities to PVL [46]. It was shown that the presence of intrauterine LPS can produce diffuse glial cell death and cavitation in fetal white matter [47]. Sherwin and Fern [48] showed that LPS can bind directly to developing white matter astrocytes and microglia to evoke rapid cell death in neighboring oligodendroglia. Astrocyte binding was particularly intense around blood vessels. In addition to direct toxicity, LPS increased the degree of acute cell death evoked by ischemia.

One of the author of the present study, Z. Ling and his colleagues (Neuropharmacology Laboratory, Department of Pharmacology, Rush University, Chairman and Project Director P. Carvey, PhD) experimentally showed that fetal brain injury by LPS also can be a risk factor for the future development of Parkinson's diseases [10,49]. The loss of DA neurons in these animals increased with age thereby mimicking the progressive pattern of cell loss seen in human Parkinson's disease [9,50]. Prenatal LPS exposure may cause disturbance in offspring brain and render DA neurons susceptible to the secondary neurotoxin insult [51].

Limitation of this study is that in vitro model of DA neurons loss caused by LPS found in vaginal fluid patients with BV can not give a full picture of all inflammatory cascade that take place in women with BV.

Our nearest goal is the study of pregnant patients' vaginal environment, tracking pregnancy outcome and newborn health.

Conclusion

Production of LPS by *P. bivia* can be an important factor in the pathogenesis of BV and subsequent complications. Further study is required to determine the range of LPS levels that can be used as an indicator for the development of adverse events for pregnant patients and for increased risk of fetal brain injury.

Level of LPS can be a marker for BV complications in reproductive age women.

Acknowledgements

We thank William Trick, MD (J. Stroger Hospital of Cook County, Chicago, IL) for statistical analysis of data. We also thank Jerry Ridal, MS (Women's Hospital of Texas, Houston, TX) for his help in culturing vaginal samples.

References

1. Strombeck L, Sandros J, Holst E, et al. *Prevotella bivia* can invade human cervix epithelial (HeLa) cells. *Apmis* 2007;115:241–51. [PubMed: 17367470]
2. Smayevsky J, Canigia LF, Lanza A, Bianchini H. Vaginal microflora associated with bacterial vaginosis in nonpregnant women: reliability of sialidase detection. *Infect Dis Obstet Gynecol* 2001;9:17–22. [PubMed: 11368254]
3. Delaney ML, Onderdonk AB. Nugent score related to vaginal culture in pregnant women. *Obstet Gynecol* 2001;98:79–84. [PubMed: 11430961]
4. Krohn MA, Hillier SL, Lee ML, Rabe LK, Eschenbach DA. Vaginal *Bacteroides* species are associated with an increased rate of preterm delivery among women in preterm labor. *J Infect Dis* 1991;164:88–93. [PubMed: 2056221]
5. Gibbs RS, McDuffie RS Jr, Kunze M, et al. Experimental intrauterine infection with *Prevotella bivia* in New Zealand White rabbits. *Am J Obstet Gynecol* 2004;190:1082–6. [PubMed: 15118646]
6. Dammann O, Leviton A. Infection remote from the brain, neonatal white matter damage, and cerebral palsy in the preterm infant. *Semin Pediatr Neurol* 1998;5:190–201. [PubMed: 9777677]
7. Edwards AD, Tan S. Perinatal infections, prematurity and brain injury. *Curr Opin Pediatr* 2006;18:119–24. [PubMed: 16601489]
8. Endo Y, Shibasaki M, Nakamura M, Takada H. Contrasting effects of lipopolysaccharides (endotoxins) from oral black-pigmented bacteria and Enterobacteriaceae on platelets, a major source of serotonin, and on histamine-forming enzyme in mice. *J Infect Dis* 1997;175:1404–12. [PubMed: 9180180]
9. Ling Z, Zhu Y, Tong C, Snyder JA, Lipton JW, Carvey PM. Progressive dopamine neuron loss following supra-nigral lipopolysaccharide (LPS) infusion into rats exposed to LPS prenatally. *Exp Neurol* 2006;199:499–512. [PubMed: 16504177]
10. Ling ZD, Chang Q, Lipton JW, Tong CW, Landers TM, Carvey PM. Combined toxicity of prenatal bacterial endotoxin exposure and postnatal 6-hydroxydopamine in the adult rat midbrain. *Neuroscience* 2004;124:619–28. [PubMed: 14980732]
11. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *American Journal of Medicine* 1983;74:14–22. [PubMed: 6600371]
12. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of Clinical Microbiology* 1991;29:297–301. [PubMed: 1706728]
13. Zariffard MR, Saifuddin M, Sha BE, Spear GT. Detection of bacterial vaginosis-related organisms by real-time PCR for *Lactobacilli*, *Gardnerella vaginalis* and *Mycoplasma hominis*. *FEMS Immunol Med Microbiol* 2002;34:277–81. [PubMed: 12443827]
14. Gayle DA, Ling Z, Tong C, Landers T, Lipton JW, Carvey PM. Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor- α , interleukin-1 β , and nitric oxide. *Brain Res Dev Brain Res* 2002;133:27–35.
15. Vu TQ, Ling ZD, Ma SY, et al. Pramipexole attenuates the dopaminergic cell loss induced by intraventricular 6-hydroxydopamine. *J Neural Transm* 2000;107:159–76. [PubMed: 10847557]
16. Nikolaitchouk N, Andersch B, Falsen E, Strombeck L, Mattsby-Baltzer I. The lower genital tract microbiota in relation to cytokine-, SLPI- and endotoxin levels: application of checkerboard DNA-DNA hybridization (CDH). *Apmis* 2008;116:263–77. [PubMed: 18397461]
17. Aroutcheva AA, Simoes JA, Behbakht K, Faro S. *Gardnerella vaginalis* isolated from patients with bacterial vaginosis and from patients with healthy vaginal ecosystems. *Clin Infect Dis* 2001;33:1022–7. [PubMed: 11528575]

18. Pybus V, Onderdonk AB. Evidence for a commensal, symbiotic relationship between *Gardnerella vaginalis* and *Prevotella bivia* involving ammonia: potential significance for bacterial vaginosis. *J Infect Dis* 1997;175:406–13. [PubMed: 9203662]
19. Thorsen P, Jensen IP, Jeune B, et al. Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: a population-based microbiologic study among 3596 pregnant women. *Am J Obstet Gynecol* 1998;178:580–7. [PubMed: 9539529]
20. Mikamo H, Kawazoe K, Sato Y, Imai A, Tamaya T. Preterm labor and bacterial intra-amniotic infection: arachidonic acid liberation by phospholipase A2 of *Prevotella bivia*. *Anaerobe* 1998;4:209–12. [PubMed: 16887644]
21. Mikamo H, Sato Y, Hayasaki Y, et al. Intravaginal bacterial flora in patients with uterine cervical cancer. High incidence of detection of *Gardnerella vaginalis*. *J Infect Chemother* 1999;5:82–85. [PubMed: 11810495]
22. Sadhu K, Domingue PA, Chow AW, Nelligan J, Cheng N, Costerton JW. *Gardnerella vaginalis* has a gram-positive cell-wall ultrastructure and lacks classical cell-wall lipopolysaccharide. *J Med Microbiol* 1989;29:229–35. [PubMed: 2787405]
23. Greenwood JR, Pickett MJ. Salient features of *Haemophilus vaginalis*. *J Clin Microbiol* 1979;9:200–4. [PubMed: 311779]
24. Sweet RL. Role of bacterial vaginosis in pelvic inflammatory disease. *Clin Infect Dis* 1995;20(Suppl 2):S271–5. [PubMed: 7548573]
25. Boggess KA, Watts DH, Hillier SL, Krohn MA, Benedetti TJ, Eschenbach DA. Bacteremia shortly after placental separation during cesarean delivery. *Obstet Gynecol* 1996;87:779–84. [PubMed: 8677085]
26. Newton ER, Wallace PA. Effects of prophylactic antibiotics on endometrial flora in women with postcesarean endometritis. *Obstet Gynecol* 1998;92:262–8. [PubMed: 9699764]
27. Cahill RJ, Tan S, Dougan G, et al. Universal DNA primers amplify bacterial DNA from human fetal membranes and link *Fusobacterium nucleatum* with prolonged preterm membrane rupture. *Mol Hum Reprod* 2005;11:761–6. [PubMed: 16254004]
28. Romero R, Gotsch F, Pineles B, Kusanovic JP. Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. *Nutr Rev* 2007;65:S194–202. [PubMed: 18240548]
29. Paul, W. *Fundamental Immunology*. Philadelphia, PA: Lippincott-Raven; 1998.
30. Mattsby-Baltzer I, Platz-Christensen J, Hosseini N, Rosen P. IL 1 beta, IL 6, TNF alpha, fetal fibronectin, and endotoxin in the lower genital tract of pregnant women with bacterial vaginosis. *Acta Obstet Gynecol Scand* 1998;77:701–706. [PubMed: 9740515]
31. Platz-Christensen JJ, Mattsby-Baltzer I, Thomsen P, Wiqvist N. Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. *Am J Obstet Gynecol* 1993;169:1161–6. [PubMed: 8238178]
32. 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]
33. Pillow JJ, Jobe AH, Collins RA, et al. Variability in preterm lamb lung mechanics after intra-amniotic endotoxin is associated with changes in surfactant pool size and morphometry. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L992–8. [PubMed: 15246974]
34. Deb K, Chaturvedi MM, Jaiswal YK. Gram-negative bacterial LPS induced poor uterine receptivity and implantation failure in mouse: alterations in IL-1beta expression in the preimplantation embryo and uterine horns. *Infect Dis Obstet Gynecol* 2005;13:125–33. [PubMed: 16126496]
35. Newton ER. The relationship between intrapartum obstetric care and chronic neurodevelopmental handicaps in children. *Reprod Toxicol* 1990;4:85–94. [PubMed: 2136032]
36. O'Shea TM, Klinepeter KL, Meis PJ, Dillard RG. Intrauterine infection and the risk of cerebral palsy in very low-birthweight infants. *Paediatr Perinat Epidemiol* 1998;12:72–83. [PubMed: 9483618]
37. Yoon BH, Park CW, Chaiworapongsa T. Intrauterine infection and the development of cerebral palsy. *Bjog* 2003;110(Suppl 20):124–7. [PubMed: 12763129]
38. Grether JK, Nelson KB. Possible decrease in prevalence of cerebral palsy in premature infants. *J Pediatr* 2000;136:133. [PubMed: 10636990]

39. Eklind S, Mallard C, Leverin AL, et al. Bacterial endotoxin sensitizes the immature brain to hypoxic--ischemic injury. *Eur J Neurosci* 2001;13:1101–6. [PubMed: 11285007]
40. Garnier Y, Coumans AB, Jensen A, Hasaart TH, Berger R. Infection-related perinatal brain injury: the pathogenic role of impaired fetal cardiovascular control. *J Soc Gynecol Investig* 2003;10:450–9.
41. Bell MJ, Hallenbeck JM, Gallo V. Determining the fetal inflammatory response in an experimental model of intrauterine inflammation in rats. *Pediatr Res* 2004;56:541–6. [PubMed: 15295096]
42. Bashiri A, Burstein E, Mazor M. Cerebral palsy and fetal inflammatory response syndrome: a review. *J Perinat Med* 2006;34:5–12. [PubMed: 16489880]
43. Stolp HB, Dziegielewska KM, Ek CJ, Potter AM, Saunders NR. Long-term changes in blood-brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat. *Eur J Neurosci* 2005;22:2805–16. [PubMed: 16324115]
44. Svedin P, Kjellmer I, Welin AK, Blad S, Mallard C. Maturation effects of lipopolysaccharide on white-matter injury in fetal sheep. *J Child Neurol* 2005;20:960–4. [PubMed: 16417842]
45. Bell MJ, Hallenbeck JM. Effects of intrauterine inflammation on developing rat brain. *J Neurosci Res* 2002;70:570–9. [PubMed: 12404511]
46. Kannan S, Saadani-Makki F, Muzik O, et al. Microglial activation in perinatal rabbit brain induced by intrauterine inflammation: detection with 11C-(R)-PK11195 and small-animal PET. *J Nucl Med* 2007;48:946–54. [PubMed: 17504871]
47. Elovitz MA, Mrinalini C, Sammel MD. Elucidating the early signal transduction pathways leading to fetal brain injury in preterm birth. *Pediatr Res* 2006;59:50–5. [PubMed: 16327009]
48. Sherwin C, Fern R. Acute lipopolysaccharide-mediated injury in neonatal white matter glia: role of TNF- α , IL-1 β , and calcium. *J Immunol* 2005;175:155–61. [PubMed: 15972642]
49. Ling Z, Gayle DA, Ma SY, et al. In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain. *Mov Disord* 2002;17:116–24. [PubMed: 11835448]
50. Carvey PM, Chang Q, Lipton JW, Ling Z. Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease. *Front Biosci* 2003;8:s826–37. [PubMed: 12957870]
51. Zhu Y, Carvey PM, Ling Z. Altered glutathione homeostasis in animals prenatally exposed to lipopolysaccharide. *Neurochem Int* 2007;50:671–80. [PubMed: 17291629]

Figure 1. Scatterplot displaying the association between the lipopolysaccharide concentration and the quantity of *Prevotella bivia* in vaginal flora.

Table 1

The quantity of *P. bivia* and the level of LPS in the vagina of patients with and without bacterial vaginosis

No-BV (59 patients)	<i>P. bivia</i> cells (in $_{10}$ log)	LPS (EU/ml)
21	0	0
16	0	6.125±3.23
16	0	26.5±4.26
4	0	54.0±13.58
1	1.9	64.0
1	2.10	128.0
BV (43 patients)		
3	0	0
1	0	32.0
1	2.11	128.0
4	3.32±0.12	128.0
4	4.32±0.17	490.0
2	5.62±0.03	520
15	6.31±0.08	3210.0±950.29
2	7.14±0.007	5030.0
9	7.52±0.13	7570.0±496.1

Table 2Association of BV with quantity of *Prevotella bivia* in the vagina

Characteristics	BV	No BV	P value
<i>P. bivia</i> < log 2 cfu/ml*	4	57	0.01
<i>P. bivia</i> ≥ log 2 cfu/ml	39	2	

* Cut point log 2 of *P. bivia* were used to classify the observed samples

Table 3

Association of BV with level of LPS in the vagina

Characteristics	BV	No BV	P value
LPS < 128 EU/ml *	9	34	0.01
LPS ≥ 128 EU/ml	58	1	

* Cut point 128 EU/ml of LPS were used to classify the observed samples