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IL-10 Modulates Placental Responses to TLR Ligands

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Abstract

Problem—Intra-uterine infections increase production of pro-inflammatory cytokines. It is unclear whether different infectious agents determine the relative expression of pro-and anti-inflammatory cytokines.

Methods of study—We compared the placental inflammatory response induced by bacterial lipopolysaccharide (LPS, endotoxin from Gram-negative bacteria) with those induced by lipoteichoic acid (LTA, a cell wall component of Gram-positive bacteria). Placental explants from term delivery were treated with either LPS or LTA, in the presence or absence of IL-10, for 24 hrs. Cytokines, prostaglandin E₂ (PGE₂) production and cyclo-oxygenase-2 (COX-2) expression were quantified.

Results—Both LTA and LPS significantly induced several cytokines with LPS eliciting more potent effects. IL-6 and IL-8 were induced to comparable levels in response to both LTA and LPS whereas monocyte chemotactic protein-1 (MCP-1) production was induced more by LTA, demonstrating a differential placental response to a specific toll-like receptor (TLR) ligand. IL-10 treatment significantly reduced most pro-inflammatory cytokines as well as PGE₂ induced by both LPS and LTA. Interestingly, IL-10 down-regulated LTA-mediated MCP1 induction, but not that mediated by LPS. Moreover, IL-10 was more effective in down-regulating PGE₂ after LPS- when compared with LTA stimulation.

Conclusions—Our results demonstrate that placental exposure to LTA and LPS appear to trigger distinct cytokine responses that can be modulated by IL-10.

Keywords

IL-10; inflammation; lipopolysaccharide; lipoteichoic acid; pre-term labor

Introduction

Pre-maturity is a major cause of neonatal morbidity and mortality, accounting for 65% of neonatal deaths and 50% of childhood neurologic disability.^{1,2} Although the mechanisms leading to pre-term birth are multifactorial, a causative association has been established with intra-uterine infection and inflammation.^{3,4} Indeed, it is estimated that infections are responsible for up to 40% of the cases of pre-term labor and birth.⁵ A spectrum of microorganisms have been isolated from the amniotic fluid from women with pre-term labor including both Gram-negative and Gram-positive bacteria.^{6,7} The ability of the immune system to recognize pathogenic organisms depends on its ability to detect conserved molecular structures known as pathogen-associated molecular patterns (PAMPs) expressed on the surface of microorganisms.⁸ Common examples of PAMPs include lipopolysaccharide (LPS) of Gram-negative bacteria and lipoteichoic acid (LTA) of Gram-positive bacteria. LTA binds to Toll-like receptor 2 (TLR2), while LPS binds to TLR4. TLR activation leads to increased levels of pro-inflammatory cytokine and chemokine activities which consequently lead to neutrophil infiltration and prostaglandin production which causes myometrial contractions and pre-term birth.⁹ Many of the biological activities are shared by both LPS and LTA.¹⁰ It has been shown that LTA can induce pre-term delivery in mice in the same manner as LPS, but the effective dose of LTA was larger than that of LPS.¹¹ The same study also showed that LTA accelerated cervical ripening and placental abruption as well as increased plasma and amniotic fluid concentrations of IL-1 β , IL-6, and tumor necrosis factor (TNF)- α .

It has been proposed that the placenta signals the onset of term and pre-term labor by up-regulating pro-inflammatory cytokines such as IL-1 β , IL-6, IL-8 and MCP-1.^{3,4,12} These pro-inflammatory cytokines are capable of inducing a complex set of biological events including myometrial changes, increased prostaglandin biosynthesis and degradation of extracellular matrix that, in turn, leads to the common pathway of labor.^{13,14} Of those cytokines at the maternal-fetal interface, IL-10 may play a central role in suppressing the activities of pro-inflammatory cytokines.¹⁵⁻¹⁷ The localized presence of IL-10 in the placenta is noteworthy, as IL-10 is the only cytokine that possesses potent anti-inflammatory and immunosuppressive activity.^{18,19} IL-10 inhibits pro-inflammatory cytokines and prostaglandin production by human chorion, decidua and placenta *in vitro*,^{20,21} as well as LPS-stimulated PGE₂ production by fetal membranes.²² We have previously demonstrated that there is a gestational age-dependent expression of IL-10 in the human placenta and that IL-10 production is significantly attenuated at term.¹⁹ These data suggest that labor is associated with withdrawal of anti-inflammatory mediators as part of an evolutionary adaptation to accelerate inflammatory processes necessary for successful labor and delivery. Furthermore, we demonstrated that infection-mediated pre-term labor in human is linked to *in utero* immune alterations caused by reduced activity of placental IL-10.²³ It has also been reported that treatment with IL-10 modulated TLR4- and TLR9-mediated pre-term birth in animal models.^{24,25} These studies clearly suggest that different inflammatory triggers can induce pre-term birth through distinct cell mediated mechanisms.

Although Gram-negative bacteria or LPS have been studied extensively as mimics for infection-mediated pre-term birth, detailed information on cytokine induction in gestational tissues following Gram-positive bacteria (and/or LTA) exposure is noticeably lacking. Likewise, few studies explored the role of IL-10 in countering the LTA-induced inflammatory mediators in gestational tissues. In this study, we compared placental inflammatory responses induced by LPS versus LTA. We hypothesize that those bacterial components produce a distinct array of placental cytokines that can be modulated by IL-10 treatment.

Methods

Tissue Collection and Processing

This study was approved by the Institutional Review Board. Placental tissue samples were collected after informed consent was obtained. Tissue samples were obtained from pregnant women who met the following inclusion criteria: (1) age between 18 and 40 years, (2) singleton pregnancy, and (3) reliable gestational age by early ultrasound. Exclusion criteria include: (1) use of recreational drugs, tobacco or alcohol during pregnancy, (2) pre-existing clinical conditions such as diabetes, hypertension, or autoimmune disease, and (3) pregnancy complications such as induced hypertension, intra-uterine growth restriction and bleeding.

Placental tissue was obtained from patients following term delivery without labor (39–41 weeks' gestation total $n = 13$) collected at the time of elective cesarean section with no rupture of fetal membranes. Additionally, term placental samples after the onset of labor (39–41 weeks' gestation; $n = 3$) were collected from normal spontaneous vaginal deliveries associated with rupture of fetal membranes occurring <8 hrs before the time of delivery. None of the term placental samples had any evidence of chorioamnionitis.

To mimic the *in vivo* scenario where paracrine interactions between different cell types and soluble factors are likely to influence the response to intrinsic signals, we used an *ex vivo* placental explant culture system^{19,23} to study the effect of LTA and LPS on placental cytokine production. Small pieces of the placenta were removed from maternal surface at different sites and placed in phosphate-buffered saline (PBS) solution and transported immediately to the lab. Before use, remaining placental tissue was separated from fetal membranes and decidual lining, rinsed with PBS and cut into 10 micron $\times$ 10 micron explants mass using Mc-Ilwain tissue chopper (Stoelting Co. Wood Dale, IL, USA). Wet tissue was weighed accurately to 300 mg and suspended in 5 mL of Dulbecco's modified Eagle's medium (DMEM) (supplemented with 5% fetal calf serum (FCS) and antibiotics) per culture plate. Dose–response curves using different concentrations of LPS or LTA (0.1–5 $\mu\text{g/mL}$) were performed using three term non-laboring placentas and three term laboring placentas. Based on PGE₂ response (see Results below), a dose of 0.5 $\mu\text{g/mL}$ was selected both for LTA and LPS (Sigma, St. Louis, MO USA) and used in all subsequent experiments with or without IL-10 (20 ng/mL;²³ R&D Systems, Minneapolis, MN, USA). The samples were then incubated in 5% CO₂ for 24 hrs at 37°C. Conditioned media was collected and stored at –80°C until analysis. Remaining explants tissues were prepared for immunohistochemistry or RNA isolation.

Immunohistochemistry

To determine the effects of LPS and LTA on cytotrophoblast apoptosis, placental tissue samples were subjected to immunohistochemical staining using specific antibodies (M30) as described.^{19,23,26} Tissues were formalin-fixed, paraffin-embedded, cut into 5- μm sections, and placed on Super Plus slides (Fisher Scientific, Pittsburgh, PA, USA). Sections were deparaffinized and rehydrated through graded alcohol using standard procedures. Endogenous peroxidase activity was quenched using a 5-min incubation step with 3% H₂O₂ in MeOH. Non-specific binding sites were blocked by incubation with 5% swine serum. Slides were incubated with M30 antibody (mouse monoclonal, CytoDEATH, Roche, NJ, USA). M30 detects the cleaved epitope of cytokeratin 18 that results from caspase-3-induced apoptosis.²⁶ All primary and secondary antibody incubations were conducted at 37°C in a humidified chamber. All slides were incubated with specific biotinylated secondary antibodies for 1 hr (Sigma). A Vectastain Elite kit (Vector Laboratories, Burlingame, CA, USA) was utilized to visualize antibody binding.

Measurement of Placental Cytokines and PGE₂

Cultured placental explants supernatants were analysed by Bio-Plex™ array system (Bio-Rad lab, Inc Hercules, CA, USA) according to the manufacturer's protocols. Cytokines that were analysed included: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, MIP-1 α , MIP-1 β , GM-CSF and MCP-1. We selected this panel of cytokines, developed by Bio-Rad, because it includes key pro- and anti-inflammatory cytokines, chemokines and growth factors that have been shown to play key role in placental immune regulation.^{3,4,6,12,13} Preliminary analysis performed with conditioned medium 'spiked' with known amounts of authentic standards, determined that the samples do not contain any compounds that interfere with the assay. The values were adjusted for dilution and sample recovery (determined from spiked media) for the final concentrations and were subjected to calculations according to standard protein values expressed in pg/mL. PGE₂ production was quantified using commercial ELISA kits (R&D Systems) according to the manufacturer's protocols. The intra- and inter-assay coefficients of variation were <10%.

Real-time PCR

Total RNA was isolated from snap frozen samples by using Tri-reagent kit as recommended by the manufacturer. Expression of COX-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was quantified by real-time PCR. RNA was isolated using RNEasy (Quiagen, Chatsworth, CA, USA). Real-time PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol and amplified on the ABI Prism 7900 sequence detection system, using GAPDH as a standard. Full-length coding sequences for the genes to be analysed were obtained from GenBank™ (National Center for Biotechnology Information). Primers were designed using Primer Express software (Applied Biosystems). Primers used were: GAPDH, tgggtacactgagcaccag and ggggtgcgtgtgtaagta; and COX-2, GCAAATCCTT-GCTGTTCCAATC and GGAGAAGGCTTCCCAGCTT.²¹

Statistical Analysis

Statistical analysis was performed using 'STATISTICA' software (STATISTICA for Windows; 1984–1994, Stat Soft Inc., Tulsa, OK, USA). Continuous data is presented as median, range and percentile. The different groups were compared by the Mann–Whitney *U*-test. The Spearman Rank Order Correlation was used to define the relationship between placental cytokines and prostaglandin under the different conditions. A *P*-value <0.05 was considered statistically significant.

Results

Selection of LTA and LPS Doses

To determine the appropriate doses of LPS and LTA to be used in our experiments, we first determined PGE₂ production in cultured placental explants collected from labor deliveries. Hence, three *labor* term placental samples were cultured for 24 hrs and the mean production of PGE₂ was determined in supernatants using ELISA (mean = 565 pg/mL). We then treated explants from 3 *non-labor* placentas with different concentrations of LPS and LTA (0.1–5 μ g/mL) and measured PGE₂ production in cultured supernatants after 24 hrs (Table I). We selected the least concentration of LPS and LTA that induced PGE₂ production in these *non-labor* placental explants in quantities similar to what was produced by explants from *labor* placentas (565 pg/mL) cultured under similar conditions. As shown in Table I, a dose of 500 ng/mL for both LPS and LTA induced PGE₂ in similar quantities as seen from placental explants from labor deliveries. As the magnitude of changes in PGE₂ production was comparable, we extrapolated that exposure of the placenta to 500 ng/mL of LPS or LTA will

induce inflammatory conditions, as suggested by PGE₂ levels, similar to what is seen in laboring placenta samples. Consequently, a dose of 500 ng/mL was selected for both LPS and LTA to be used in our subsequent experiments.

LPS or LTA Did Not Induce Cytotrophoblast Apoptosis

To determine whether LPS or LTA (500 ng/mL) induced cytotrophoblast apoptosis, cultured placental explants were collected after 24 hrs, fixed and stained using monoclonal antibody (M30) directed against a neo-epitope of cytokeratin 18. This method was shown to be of advantage over other tests for the detection of trophoblast cell apoptosis.²⁶ Immunohistochemistry did not show increased staining in sections treated with LPS or LTA when compared with control (Fig. 1).

Cytokine Expression in Cultured Placental Supernatants in Response to LTA and LPS

Cytokine production was analysed in the supernatants of placental explant cultures incubated for 24 hrs after treatment with LPS or LTA ($n = 10$). IL-2, IL-4, IL-5 and IL-7 were not detected. Fold increases in cytokine means were compared with the nontreatment group (control). Both LTA and LPS induced five cytokines (IL-1 β , MIP-1 α , MIP-1 β , GM-CSF and IL-10), however, the fold increase was more noticeable after LPS treatment when compared with LTA (Table II). Two other cytokines (IL-6 and IL-8) increased to a similar magnitude following treatment with LTA or LPS. Although not statistically significant, LTA trended to induce more MCP-1 production when compared with LPS treatment demonstrating a differential cytokine placental response to these TLR ligands. The effects of LPS and LTA on placental cytokine production were further shown in Fig. 2. Although there was inter-individual variability among subjects as shown, each placental sample ($n = 10$) responded to LTA or LPS treatments in a similar pattern (Fig. 2, left). IL-6 and IL-8 were induced in comparable magnitudes in response to LTA and LPS stimulation. The rest of the cytokines were induced by LPS in more significant quantities when compared with LTA with the exception of MCP-1, which was produced in more significant amounts in response to LTA. To demonstrate the role of IL-10 in modulating LTA and LPS effects on placental cytokine production, placental explants from non-laboring placentas were treated with LPS or LTA in the presence or absence of IL-10 (20 ng/mL). Cytokine production was evaluated from supernatants as described before. As seen in Fig. 2 (right), IL-10 significantly reduced LPS/LTA-induced cytokine production for IL-1 β , MIP1 α , MIP1 β , IL-6 and IL-8 and increased production of the pro-pregnancy cytokine GM-CSF. Interestingly, IL-10 significantly down-regulated MCP1 induction after LTA- but not LPS stimulation. Both LTA and LPS significantly increased IL-10 production, although with higher magnitude using LPS.

Role of IL-10 in Modulating LTA and LPS Effects on Placental PGE₂ Production and COX-2 Expression

To further investigate the role of LTA and LPS in inducing placental PGE₂ and COX-2 expression, supernatants and placental samples ($n = 10$) were collected after 24 hrs of treatment with LTA or LPS (500 ng/mL) with or without IL-10 (20 ng/mL) as described in Materials and Methods. The PGE₂ concentration in conditioned medium was significantly increased in response to both LTA and LPS. Although IL-10 treatment appeared to inhibit PGE₂ production by placental tissues after LTA and LPS stimulation, results did not reach statistical significance with un-stimulated samples or LTA (Fig. 3). Next, COX-2 mRNA expression was evaluated by semi-quantitative RT-PCR analysis (Fig. 4). Similar to PGE₂ data, IL-10 treatment resulted in significant inhibition of COX-2 mRNA levels after LPS stimulation. Although IL-10 reduced COX-2 expression after LTA stimulation, it did not reach statistical significance (Fig. 4).

Discussion

Intra-uterine infections caused by bacteria are considered to be the leading cause of pre-term birth.⁵ Although LPS is frequently used as a model of infection-mediated pre-term labor, other species of microorganisms are implicated in the pathogenesis of infection-mediated pre-term labor including *Mycoplasma hominis*, *Streptococcus agalactiae*, *Escherichia coli*, *Fusobacterium species*, and *Gardnerella vaginalis*.^{27,28} Activating different TLRs by diverse microorganism components, trigger downstream pathways, leading to the induction of distinctive, but overlapping pro- and anti-inflammatory cytokine profiles.^{29,30} Delicate balance between anti- and pro-inflammatory mediators is essential for maintaining immune function against invading organisms as well as protecting the body from over production of pro-inflammatory mediators. A solid body of evidence indicates that cytokines play a central role in the mechanisms of inflammation/infection-induced pre-term parturition.³⁻⁶ It has been proposed that the placenta signals the onset of term and pre-term labor by up-regulating pro-inflammatory cytokines such as IL-1 β , IL-6, IL-8 and MCP-1^{3,4,12} or down-regulating the anti-inflammatory cytokine IL-10.^{19,23}

Several studies have demonstrated the presence and regulation of TLR2 and TLR4 in the villous and the intermediate trophoblasts.^{31,32} To better understand pathogen–host interactions, the placental cytokine response to different TLR ligands was characterized in our study. Pro- and anti-inflammatory cytokine profiles after LPS stimulation (TLR4 ligand, representative of Gram-negative organisms) when compared with LTA (TLR2 ligand representative of Gram-positive organisms) were evaluated. Other studies demonstrated the ability of TLR2 and TLR4 stimulation to induce pro-inflammatory cytokines IL-6 and IL-8 production in the placenta cultures³¹ as well as COX-2 and PGE₂ in trophoblast cells via NF-kappa B and MAP kinase pathways.³³ Our results indicate that LTA and LPS induced mainly pro-inflammatory cytokines along with IL-10, possibly to exert self-regulatory control. In addition, these two diverse inflammatory triggers exhibited potency in inducing distinct cytokines, suggesting that different inflammatory triggers may elicit diverse responses for programming of pre-term labor and birth. It should be noted that LPS molecular weight is much heavier than LTA. Therefore, the stimulation of cytokine production by LPS was done with fewer molecules indicating that on a molecular basis LPS may be even more potent stimulator of pro-inflammatory cytokines when compared with LTA than what our study suggests (done on an equal per mass basis).

IL-10 is believed to be a key cytokine for the maintenance of pregnancy by tightly regulating pro-inflammatory cytokines at the maternal-fetal interface. Several studies have demonstrated that IL-10 can down-regulate COX-2 and PGE₂ production. Pomini et al.³⁴ reported that IL-1 β stimulates COX-2 expression and PGE₂ production in cultured placental trophoblasts that was reversed after IL-10 treatment. Similarly, IL-10 was able to inhibit the output of PGE₂ from intact fetal membranes under basal and LPS-stimulated conditions, and there was a parallel decrease in the expression of mRNA for COX-2.³⁵ We have previously demonstrated that IL-10 production is significantly reduced in the placenta of patients at term compared with that from first- and second trimester tissues, suggesting that down-regulation of IL-10 is a physiologic event that favors an inflammatory state leading to the onset of labor.¹⁹ IL-10 has also been implicated in the control of pre-term parturition associated with inflammation.²³⁻²⁵ Our previous results demonstrated that infection-mediated pre-term labor in humans is linked to *in utero* immune alterations caused by reduced activity of placental IL-10.²³ However it is unclear if IL-10 is effective in altering placental immune response to different organisms in the context of pre-term labor. In this study, we demonstrated that LPS induces placental IL-10 levels to a more significant degree when compared with LTA. Furthermore, our results demonstrated that IL-10 treatment considerably reduced most pro-inflammatory cytokine production as well as PGE₂ induced

by both LPS and LTA, suggesting that IL-10 may play a crucial role in controlling pathologic events associated with infection caused by different organisms in the placenta. Interestingly, IL-10 significantly down-regulated MCP-1 induction after LTA and not LPS stimulation, however IL-10 was more effective in down-regulating PGE₂ after LPS- in relation to LTA stimulation. This indicates that different organisms appear to activate different signaling pathways, triggering distinct placental cytokine responses.

Taken together, the results of this study show that LPS and LTA induce distinct but overlapping pro-and anti-inflammatory cytokine responses. We speculate that IL-10 as a tocolytic agent may prove to be a potent effector in infection-mediated pre-term labor and birth scenario. These results will be further used as the basis for comparative studies of the placental immune responses to various organisms (including *Ureaplasma*, *Mycoplasma* as well as heat-inactivated Gram-negative and -positive organisms). Such studies may provide a better understanding of the distinct placental pathways activated by various organisms, and provide further clues to develop more successful immunotherapy and possible vaccination strategies for prevention of pre-term labor.

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Fig. 1.

To identify apoptotic cytotrophoblasts in explant tissue, immunohistochemical detection of M30 in placental tissues was performed as described in Materials and methods. M30 staining was localized in the trophoblast layer and was similar in sections treated with lipopolysaccharide (LPS) or lipoteichoic acid (LTA) when compared with control. Shown are results that are representative of ten experiments (magnification, 40 $\times$).

Fig. 2.

Analysis of cytokine production in placental explants cultures. Placental tissues were minced, treated with lipopolysaccharide (LPS), lipoteichoic acid (LTA) or IL-10 and cultured for 24 hrs. Conditioned medium was analysed for cytokine levels using Bio-Plex™ array system. The graphs on the left demonstrate the cytokine response of every sample ($n = 10$) to either LTA or LPS. The graphs on the right demonstrate the cytokine response to LTA and LPS with or without IL-10. Shaded box areas represent 25th-75th percentile intervals with the median represented by the horizontal line within the box. Vertical lines represent 10th-90th percentile intervals. Note that MIP-1 α , MIP-1 β and GM-CSF are plotted on a log scale as there was significant variability in cytokine levels between samples that were treated with bacterial products when compared with un-stimulated samples. The data represents cytokine measurements from 10 independent tissue samples.* P -value <0.05 for the difference between means.

Fig. 3.

Placental explants ($n = 10$) from term deliveries were incubated for 24 hrs in the absence or presence of lipoteichoic acid (LTA), lipopolysaccharide (LPS) or IL-10. Culture supernatant was collected and analysed for PGE₂ production by ELISA as described in Materials and methods. Explants produced significant quantities of PGE₂ even without stimulation. Both LTA and LPS induced PGE₂ production in significant amounts when compared with untreated samples (P -value <0.05). Note that IL-10 treatment resulted in significant inhibition of PGE₂ production (with or without LPS co-stimulation P -value <0.05). Interestingly, IL-10 reduced PGE₂ production after LTA stimulation but did not reach statistical significance. Shown are means $\pm$ S.E.M. for experiments conducted on 10 placentas. * P -value <0.05 for the difference between means.

Fig. 4.

Analysis of COX-2 mRNA expression in placental tissues ($n = 10$) in response to lipoteichoic acid (LTA), lipopolysaccharide (LPS) or IL-10 treatments. Total RNA was extracted, cDNA generated, and gene expression quantified by real-time PCR. Each bar represents the mean $\pm$ S.E.M. for experiments conducted on 10 placentas. Similar to PGE₂ data, IL-10 treatment resulted in significant inhibition of COX-2 expression after LPS stimulation. *Significant difference ($P < 0.05$) from LPS treatment. Although IL-10 reduced COX-2 expression in un-stimulated samples as well as after LTA stimulation, it did not reach statistical significance.

Table I

Non-laboring placental explants were cultured with different doses of lipopolysaccharide (LPS) and lipoteichoic acid (LTA) for 24 hrs. PGE₂ concentrations were measured. As observed, a dose of 500 ng/mL was the least dose of LPS and LTA that induced PGE₂ production in quantities similar to what was observed in laboring placental explants (mean = 565 pg/mL). PGE₂ production in non-labor placentas were similar to laboring placentas when treated with LPS at a dose of 500 ng/mL ($P = \text{NS}$) and LTA at a dose of 500 ng/mL ($P = \text{NS}$)

Dose	Placenta 1	Placenta 2	Placenta 3	Mean PGE ₂ (pg/mL)
Control	251	344	288	294
LPS 100 ng/mL	551	431	500	494
LPS 500 ng/mL	596	541	590	575
LPS 1 µg/mL	683	541	599	607
LPS 2 µg/mL	673	457	602	577
LPS 5 µg/mL	636	457	580	557
LTA 100 ng/mL	586	446	430	487
LTA 500 ng/mL	636	491	589	571
LTA 1 µg/mL	729	392	568	553
LTA 2 µg/mL	656	499	588	571
LTA 5 µg/mL	646	532	600	592

Table II

Placental explants were cultured for 24 hrs with or without lipopolysaccharide (LPS) and lipoteichoic acid (LTA). Supernatants were assayed for cytokine production. Fold increases in mean cytokine production when compared with untreated samples are shown. Four cytokines (IL-1 β , MIP-1 α , MIP-1 β , and IL-10) were induced in greater amounts after LPS stimulation when compared with LTA. There was a trend for increased production of GM-CSF after LPS stimulation when compared with LTA but was not statistically significant. In contrast LTA trended to induce more MCP-1 production when compared with LPS but was also not statistically significant. Both LPS and LTA induced IL-6 and IL-8 in similar magnitude.

	LTA	LPS
IL-1b	11.9	37.7 *
MIP1- α	42.4	82.1 *
MIP1- β	22.1	31.0 *
IL-10	12.7	43.9 *
GM-CSF	26.5	39.1
MCP-1	6.3	3.9
IL-6	5.0	5.8
IL-8	4.1	4.8

* Significant difference (P -value <0.05) for fold induction between LPS and LTA.