

Short Communication



Effect of Intrauterine Administration of Lipopolysaccharide (LPS) on the Transcriptional Profile of IL2, TNF α and IFN γ in Mice

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Abstract | The effect of intrauterine (IU) infusion of lipopolysaccharide (LPS) on some of the proinflammatory cytokine was studied in a mice model. Female mice of nine week age (n=24) were divided into two equal groups; one group received LPS (*E. coli* O127:B8) 25 μ g in 100 μ l PBS intrauterine while the other received PBS 100 μ l as vehicle. Immunokinetic study was done 12 and 24hrs post-LPS treatment by sacrificing 6 mice from each group at each time and relative expression of cytokines (IL2, TNF α and IFN γ) by real time PCR in the uterine tissue. Apparent up-regulation of IL2 cytokine mRNA expression at 12h (2-fold) and 24h (37-fold, P<0.05) was recorded in LPS-infused mice. Similarly, the expression of TNF α mRNA was elevated by 6 and 35-fold at 12 and 24h post infusion, respectively. The increase in the expression of IFN γ was found only at 12h (1.8-fold, P<0.05) in LPS infused mice. It is concluded that intrauterine administration of LPS in the mice elevated the expression of proinflammatory cytokines.

Keywords | Endometritis, Cytokine, Lipopolysaccharide, Mice, Intrauterine

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Escherichia coli is the most common non-specific, opportunistic pathogens that contaminate the postpartum uterus, thereby causing endometritis. The pathogenicity of *E. coli* is dependent on certain antigens that are essential for adhesion to epithelial cells, motility and cell wall associated endotoxin (like lipopolysaccharide, LPS) (Sheldon et al., 2010). Heat-killed *E. coli* and LPS initiate similar inflammatory responses by the endometrial cells vis-à-vis expression of inflammatory cytokines and enzymes of prostaglandin synthesis (Herath et al., 2006b), suggesting the importance of LPS in *E. coli* endometritis. Host cells recognize LPS via surface receptors, leading to initiation of downstream signaling that stimulate the production of nitric oxide, pro-inflammatory cytokines and chemokines (Herath et al., 2006b). These cytokines then further provide a positive feedback loop to increase the immune cell mobilization and further secretion of pro-inflammatory mediators. In addition to LPS, presence of virulent factor fim H gene of *E. coli* on postpartum day 1-3 was associat-

ed with a significantly increased risk of subsequent postpartum endometritis in the cow (Bicalho et al., 2012). A few studies have been carried out in mice with respect to the cytokine profile vis-à-vis the effect of LPS on uterine receptivity and embryonic development (Deb et al., 2005; Aisemberg et al., 2007). When endometrial specific strain of *E. coli* (cluster 4 O73:H16) and its purified LPS were infused into the uterine lumen of C57BL6 mice, it developed septic metritis with neutrophilic infiltration within 24 h (Sheldon et al., 2010). Recently, Hasan et al. (2013) demonstrated development of endometritis in the mice following intracervical inoculation of *E. coli* @ 10⁴ cfu/mL after 24 h of treatment. In the present study, the relative expression of selected cytokines in uterine tissue subsequent to intrauterine infusion of *E. coli* LPS was studied.

Female mice of nine week age (n=24) were divided into two equal groups. One group received LPS (*E. coli* O127:B8) 25 μ g in 100 μ l PBS intrauterine while the other

Table 1: Primers used to study the expression profile of genes coding for different cytokines in the mice

Gene	Sequence (5'-3')	Primer length (bp)	Amplicon size (bp)
IL-2	F: CCTGAGCAGGATGGAGAATTACA	23	141
	R: TCCAGAACATGCCGCAGAG	19	
TNF- α	F: CATCTTCTCAAAATTCGAGTGACA A	25	175
	R: TGGGAGTAGACAAGG TACAACCC	23	
IFN- α	F: TCAAGTGGCATAGATGTGGAAGAA	24	92
	R: TGGCTCTGCAGGATTTTCATG	21	
β -actin	F: AGAGGGAAATCGTGCGTG AC	20	148
	R: CAATAGTGATGACCTGGCCGT	21	

administered PBS 100 μ l as vehicle. The stage of estrous cycle was determined based on vaginal cytology (Byers et al., 2012). Mice in estrus phase were secured in dorsal recumbency and the LPS or PBS was infused intrauterine. The mice were sacrificed at 12 or 24h interval and the uterine tissue were collected. A total of 1g tissue was weighed and used for total RNA extraction using Trizol reagent following the standard protocol. Extracted RNA was reverse transcribed to synthesize cDNA and polymerase chain reaction was done using specific primer sets (Overbergh et al., 1999) of target genes (Table 1). The PCR products were resolved in 2% (w/v) agarose gel along with a 100 bp DNA marker (Figure 1). The relative expression of IL2, TNF α and IFN γ in the uterine tissue of mice was evaluated by real-time PCR using SYBR green chemistry (Applied Biosystems). β -actin served as endogenous control gene while PBS treated group served as calibrator. The relative fold-change for each cytokine gene at 12 and 24h post-LPS infusion was analyzed by non-parametric Mann-Whitney U test.

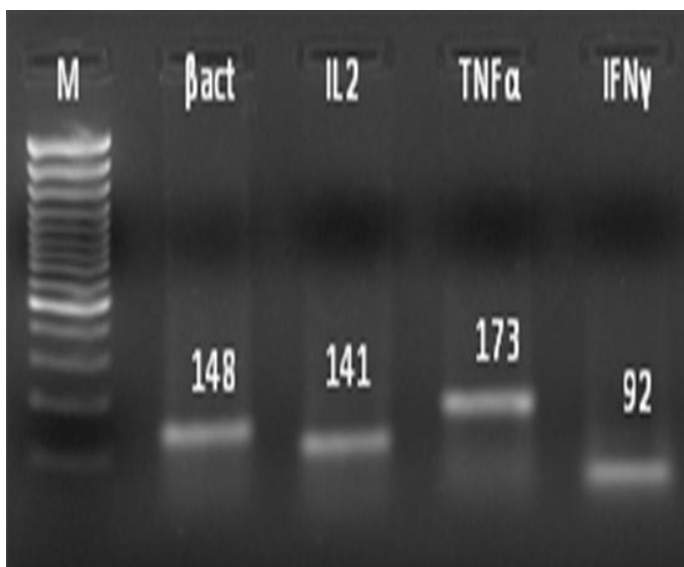


Figure 1: RT-PCR products of β -actin, IL2, TNF α and IFN γ resolved in 2% agarose gel

In this study, IL2 and IFN γ expression were significantly ($P < 0.05$) up-regulated at 24h and 12h post-LPS infusion, respectively as compared to control. However, a non-sig-

nificant elevation in expression of TNF α at 12 and 24h post LPS infusion relative to control was recorded (Table 2). A numerical increase in the fold expression of the TNF α without significance might be due to low statistical power consequent to high heterogeneity.

Table 2: Real-time transcriptional profile of the cytokine genes in the uterine tissue of LPS- infused mice relative to control

Cytokine gene	Time Post-LPS infusion (h)	Fold increase (n=6)	P value
IL2	12	2.38 \pm 1.36	0.647
	24	36.75 \pm 23.48*	0.037
TNF α	12	6.258 \pm 3.00	0.647
	24	35.14 \pm 17.64	0.431
IFN γ	12	1.81 \pm 0.02*	0.022
	24	0.98 \pm 0.49	0.115

* Significance at 95% level ($P < 0.05$)

LPS is the main pathogenic ligand of *E. coli* and a strong activator of innate immune response (Herath et al., 2006a). Following infection, an immune response is initiated by cell surface Toll-like receptor-4 (TLR4), CD14 and MD2 (Herath et al., 2006b; Herath et al., 2009), that detect pathogens and in turn trigger signaling pathways that activate molecules (transcription factors) that controls the induction of a proinflammatory immune response through activation of genes encoding cytokines (IFN γ , IL2 and TNF α) and chemokines (Bonizzi and Karin, 2004). It is suggested that LPS can trigger TNF α release by interferon-primed macrophages and induce IFN γ production, resulting in an additive effect on the production of TNF α . In addition, a higher endometrial expression of TLR4 in clinical endometritis was reported in buffaloes (Loyi et al., 2013). It is reported that treatment of bovine granulosa cells in culture with LPS adversely affected oocyte maturation and increased IL6 transcripts (Bromfield and Sheldon, 2011) and steroidogenic capacity through TLR4 pathway (Price et al., 2013).

The elevated levels of TNF α and IFN γ in our study are in agreement with the reports of Nandi et al. (2010) who re-

ported that bacterial LPS in the bloodstream induced production of inflammatory cytokines such as TNF α , IL1 β and IFN γ . TNF is secreted from a variety of gestational cells upon stimulation with bacteria or lipopolysaccharides *in vitro* including placental fragments, amnion, and chorio-decidua (Sato et al., 2003). Herath et al. (2006b) also described the increased expression of TNF α in the cultured endometrial epithelial and stromal cells stimulated *in vitro* with LPS. However, Yang et al. (2009) did not observe a classical predominance of Th1 cytokine in the plasma of mouse of pre-term labour following intrauterine infusion of LPS.

Upregulation of IL2, IFN γ and TNF α transcripts in the uterine tissue of mice following LPS infusion in the present study indicates uterine inflammation. Intravaginal inoculation of mice with specific bovine uterine isolates of *E. coli* will further support the present observation. The efficacy of novel intrauterine drugs for the treatment of endometritis in the bovine may be tested using this model.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

The work was carried out by Tumnyak Loyi as part of her PhD thesis under the guidance of Harendra Kumar and Sukdeb Nandi. Manas Kumar Patra and Rafiqul Islam assisted in designing of experiment, sample collection, and analysis of result. Krishnaswamy Narayanan assisted in statistical analysis and preparation of the manuscript.

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